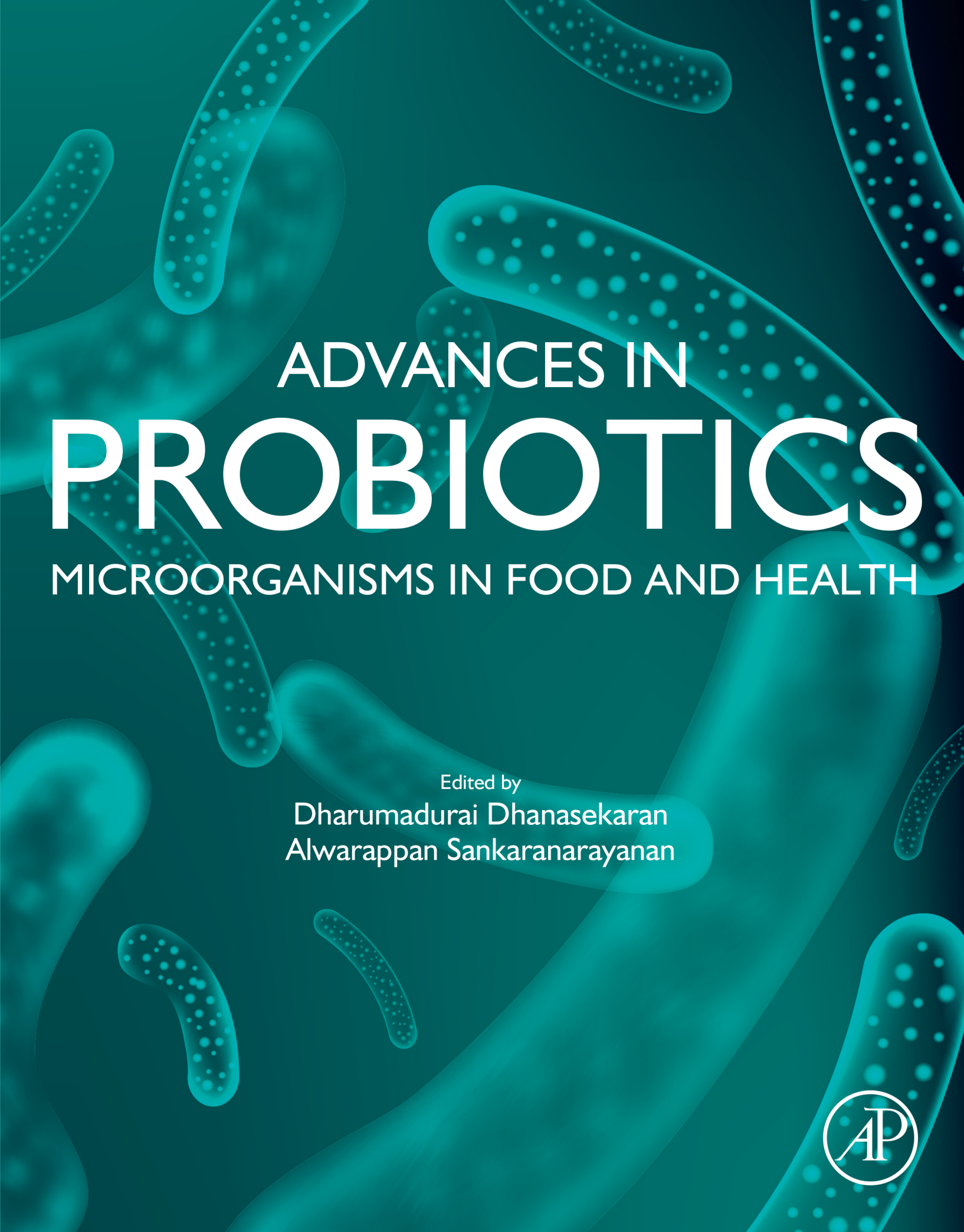

ADVANCES IN
PROBIOTICS
MICROORGANISMS IN FOOD AND HEALTH
Edited by
Dharumadurai Dhanasekaran
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Advances in Probiotics

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Advances in Probiotics

Microorganisms in Food and Health

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ACADEMIC PRESS

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Academic Press is an imprint of Elsevier
125 London Wall, London EC2Y 5AS, United Kingdom
525 B Street, Suite 1650, San Diego, CA 92101, United States
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom

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Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

ISBN: 978-0-12-822909-5

For information on all Academic Press publications
visit our website at https://www.elsevier.com/books-and-journals

Publisher: Charlotte Cockle
Acquisitions Editor: Patricia Osborn
Editorial Project Manager: Emerald Li
Production Project Manager: Sojan P. Pazhayattil
Designer: Miles Hitchen

Typeset by Thomson Digital



Contents

Contributors xv
Editors Biography xix
Foreword xxi
Preface xxiii

Part I
Probiotics Microorganisms

1 Probiotic Microorganisms and Their Benefit to Human Health

Sabina Fijan, Jessica A. ter Haar and László Varga

1 Introduction 3
2 Most common detection methods and assays of probiotic microorganisms 4
2.1 In vitro assays 4
2.2 In vivo animal assays 6
2.3 Clinical studies 6
3 Probiotic microorganisms and their recently reported health effects 6
3.1 Probiotics, the genus *Lactobacillus*, and novel lactobacilli genera 6
3.2 Probiotics and the genus *Bifidobacterium* 10
3.3 Genera of probiotic lactic acid bacteria other than lactobacilli 12
3.4 Genera of other probiotic bacteria and yeasts 13
3.5 Novel or next-generation probiotics 14
4 Discussion and conclusions 15
References **15**

2 Selection Criteria for Identifying Putative Probiont

Başar Uymaz Tezel, Pınar Şanlıbaba, Nefise Akçelik and Mustafa Akçelik

1 Introduction 23
2 Probiotic microorganisms 24
3 Requirements for the selection of probiotic strains 25
3.1 Survival during gastrointestinal transit 26
3.2 Adhesion to gut cells 26
3.3 Antipathogenic activity 27
4 Safety assessments 28
4.1 Virulence factors 29
4.2 Antibiotic resistance 29
4.3 Taxonomy/Identification 30
5 Technological requirements 30
6 Conclusion 31
References **32**

3 Simulated Gastrointestinal System to Assess the Probiotic Properties Modified to Encapsulation of Probiotics and Their Survival Under Simulated Gastrointestinal System

Ifra Hassan, Adil Gani and Zanoor Ul Ashraf

1 Introduction 37
2 The gastrointestinal (GI) tract 38
2.1 Microbiota of the adult GI tract 38
2.2 Characteristics of the GI tract for probiotic delivery 38
3 Encapsulation technologies for probiotics 39
3.1 Selecting the biomaterials for microencapsulation 40
4 Selecting the in vitro conditions for cells release 41
5 Survival of entrapped LCS in simulated gastrointestinal conditions 42
6 Conclusion 42
References **43**

4 Next-Generation Probiotics

Manorama Kumari and Anusha Kokkiligadda

1 Introduction 45
2 Next-generation probiotics 46
2.1 Need for next-generation probiotics 46
3 Candidates for next-generation probiotics 47
3.1 *Akkermansia muciniphila* 47
3.2 *Bacteroides fragilis* *55*
3.3 *Faecalibacterium prausnitzii* *58*
3.4 *Eubacterium hallii* *64*
3.5 *Parabacteroides goldsteinii* *66*

4 Safety assessment of next-generation probiotics 68
5 Application of next-generation probiotics 69
5.1 Current development 69
5.2 Technical challenges 69
5.3 Regulatory challenges 70
6 Conclusion 71
References 71

5 Edible Mushrooms: A Promising Bioresource for Prebiotics

Karthiyayini Balakrishnan, Dharumadurai Dhanasekaran, Vinothini Krishnaraj, A. Anbukumaran, Thirumurugan Ramasamy and Muthuselvam Manickam

1 Introduction 81
1.1 Mushrooms as food values 83
1.2 Energy value of mushrooms 83
1.3 Mushrooms in India 84
1.4 Bioactive compounds of mushrooms 84
1.5 Low-molecular and high-molecular weight compounds in mushroom 84
1.6 Importance of prebiotics 86
1.7 Prebiotic concept 86
1.8 Prebiotic index 86
1.9 Benefits of prebiotics 87
1.10 Properties of prebiotics 87
1.11 Characteristics of ideal prebiotics 88
1.12 Mechanism of prebiotics 88
1.13 Potential immunomodulatory mechanism of prebiotics 89
1.14 Gastrointestinal effects of prebiotics 89
1.15 Effects of prebiotic in gastrointestinal 90
1.16 Mushrooms as a promising prebiotic 90
1.17 Criteria of prebiotics 92
1.18 Role of mushrooms as prebiotics 92
2 Conclusions 93
References 94

Part II
Omics approaches in Probiotics

6 Genetic Modification and Sequence Analysis of Probiotic Microorganisms

Mustafa Akçelik, Nefise Akçelik, Pınar Şanlıbaba and Başar Uymaz Tezel

1 Introduction 101
2 Sequence analyses 102
3 Genetic engineering applications on probiotic strains 103
3.1 Food-grade vectors 103
3.2 DNA transfer 105
3.3 Genetic stability 106
3.4 Expression systems 106
3.5 Protein transport 107
4 Use of CRISPR-Cas systems 107
5 Systems biology approaches 108
6 Biosafety 109
7 Conclusion 110
References 110

7 Biosynthetic Gene Cluster Analysis in *Lactobacillus* Species Using antiSMASH

Manickasamy Mukesh Kumar and Dharumadurai Dhanasekaran

1 Introduction 113
2 In vitro and in vivo studies on beneficial effects of probiotics 114
2.1 Bowel diseases and the immune system 114
2.2 Dermal health 114
2.3 Dental caries 114
3 Modulation of gut–brain axis by probiotics 114
4 Materials and methods 114
4.1 Selection of genes from GenBank 114
4.2 Scrutiny of dataset 115
5 Secondary metabolite clusters identification using antiSMASH 115
6 Phylogenetic analysis of genes 115
7 pH concentration 115
8 Result and discussion 115
8.1 Selection of genes from GenBank 115
8.2 Scrutiny of dataset 115
9 Secondary metabolite clusters identification using antiSMASH 116
10 Phylogenetic analysis of genes 117
11 pH concentration 118
12 Conclusion 118
References 119

8 Probiotic Polysaccharides as Toll-Like Receptor 4 Modulators—An In Silico Strategy

T. Muthu Kumar and K. Ramanathan

1 Introduction 121
2 Methodology 122
2.1 Dataset 122
2.2 Protein and ligand preparation 123
2.3 Molecular docking and prime MM/GMSA 123
2.4 ADME analysis 123
2.5 Molecular dynamics 123

3 Results and discussion 124
3.1 Molecular docking 124
3.2 ADME analysis 127
3.3 Molecular dynamics 127
4 Conclusion 132
References 132

Part III
Quality and Nutrition of Probiotics

9 Prebiotics Mechanism of Action: An Over View

Shanmugaraj Gowrishankar, Arumugam Kamaladevi and Shunmugiah Karutha Pandian

1 Introduction 137
2 Mechanism of prebiotics in treating constipation 138
3 Mechanism of action of prebiotics in maintaining intestinal pH 138
4 Mechanism of action of prebiotics in maintaining lipid metabolism 138
5 Mechanism of action of prebiotics as anticarcinogenic agents 139
6 Mechanism of action of prebiotics in immunomodulation 139
7 Mechanism of action of prebiotics in preventing necrotizing enterocolitis (NEC) 140
8 Mechanism of action of prebiotics in preventing diabetes 140
9 Mechanism of action of prebiotics in preventing bowel diseases 140
10 Mechanism of prebiotics in improving nutritional absorption 141
11 Mechanism of action of prebiotics in maintaining nervous system 142
12 Mechanism of action of prebiotics in preventing autism 142
13 Mechanism of action of prebiotics in preventing hepatic encephalopathy 142
14 Mechanism of action of prebiotics in preventing skin diseases 143
15 Mechanism of action of prebiotics in preventing cardiovascular diseases 143
16 Conclusion 143
References 143

10 Synbiotics in Nutrition

Nazar Reehana, Mohamed Yousuff Mohamed Imran, Nooruddin Thajuddin and Dharumadurai Dhanasekaran

1 Introduction 149
2 Synbiotics 149
3 Synbiotic selection criteria 150
4 Synbiotics in use 150
5 Mechanism of action of synbiotics 150
6 Synbiotics for humans 151
7 Synbiotics and their outcomes on human health in clinical studies 152
8 Metabolic syndrome 152
9 Inflammatory bowel disease 152
10 Diarrhea 153
11 Irritable bowel syndrome 154
12 Colon cancer 154
13 Kidney and liver disease 155
14 Synbiotics for animals 155
15 Synbiotic therapy 156
16 Application of synbiotics 156
17 Commercial synbiotics: obstacles, challenges, and future prospects 157
18 The safety issue of synbiotics 158
19 Conclusion 158
References 158

11 Role of Probiotic Microbes Exerting Nutritional Properties

T. Savitha and Alwarappan Sankaranarayanan

1 Introduction 163
2 Overview about probiotic foods 164
3 Probiotic food products 167
3.1 Dairy-based probiotic foods 169
3.2 Nondairy probiotic products 171
4 Microbial role in probiotic foods and nutritional properties 172
5 Probiotic food and its clinical significance—human health perspectives 174
5.1 Antimicrobial potential of probiotic foods 174
5.2 Antiinflammatory activity of probiotic food and human health 174
5.3 Antiobesity and probiotic foods 175
5.4 Probiotic food and antidiabetic activities 175
5.5 Anticancer properties of probiotic foods 175
5.6 Probiotic foods and its effect on brain and CNS 175
6 Role of probiotics in dietary supplements 176
7 Global emergence of probiotic foods 176
8 Nutraceutical importance of probiotic foods 177
9 Future perspectives of probiotic foods 177
10 Conclusion 177
References 178

Part IV
Probiotics in Health and Diseases

12 Probiotic Microorganism: A Promising and Innovative Tool for Cancer Prevention and Therapy

Nabendu Debnath, Ashok Kumar Yadav and Ashish Tyagi

1 Introduction 187
2 Chronic inflammation is a major oncogenic stimulant 188
3 Mechanism of action of probiotics against cancer 189
3.1 Modulation of oxidative stress 189
3.2 Effects on carcinogen/genotoxic compounds 190
3.3 Effects on bacterial enzymatic activity 190
3.4 Immunomodulatory functions against cancer 191
3.5 Effects of probiotics on tumor microenvironment 191
3.6 Effects of probiotics on apoptosis 191
4 In vitro studies of probiotics on cancer 192
5 In vivo studies of probiotics on cancer 193
6 Probiotics and gastrointestinal (GI) cancer 195
6.1 Probiotics against colorectal cancer (CRC) 195
6.2 Probiotics against gastric cancer (GC) 195
7 Afterword 196
References 196

13 Psychobiotics: A Newer Approach Toward the Treatment of Neurodevelopmental Disorders

Tamalika Chakraborty, Jeenatara Begum, Dipanjan Mandal and Abhijit Sengupta

1 Introduction 203
2 Gut microbiota as psychobiotics 204
3 Prebiotics for psychobiotics 205
4 Psychophysiological effects of psychobiotics 205
5 Microbes–brain signaling 206
6 Mind-enteric nervous system interaction 207
7 Vagal signaling 207
8 Short-chain fatty acids, gut hormones, and bacteria-derived blood metabolites 208
9 Microbes immune interactions 209
10 Neuropsychological disorders 210
11 Metabolic disorder 211
12 Gastro Intestinal Issue 212
13 Regulation of microbiota and possibilities for treatment 212
14 Conclusion 213
References 213

14 Probiotics, Diet, and Gut Microbiome Modulation in Metabolic Syndromes Prevention

Fred Kwame Ofosu, Dylis-Judith Fafa Mensah, Eric Banan-Mwine Daliri, Byong-Hoon Lee and Deog-Hwan Oh

1 Metabolic syndrome 217
1.1 Metabolic syndrome and diet 218
2 Unveiling the potential of probiotics 221
2.1 Probiotics and dysbiosis 222
3 Gut microbiota in obesity 222
3.1 Probiotics and obesity 223
4 Probiotics and cardiovascular diseases 227
5 Conclusion 228
References 228

15 *Bacillus* Species—Elucidating the Dilemma on Their Probiotic and Pathogenic Traits

Loganathan Gayathri and Athirathinam Krubha

1 Introduction 233
2 Advantages of sporeformers in the gut and food chain 234
3 Probiotic attributes of *Bacillus* species 234
4 Synbiotics of *Bacillus* sp. 237
5 The rationale to use synbiotics 237
6 Mechanism of action of *Bacillus* probiotics 239
7 Mechanism 1—antimicrobial activity 239
8 Mechanism 2—interaction with intestinal and immune cells 240
9 Commercially available *Bacillus* probiotics 240
10 Pathogenic attributes of *Bacillus* sp. 241
11 *Bacillus* probiotics—safety 242
12 Conclusion 243
References 243

16 Probiotic Fortified Seaweed Silage as Feed Supplement in Marine Hatcheries

Charles Santhanaraju Vairappan

1 Introduction 247
2 Issues in aquaculture hatcheries 247
3 Use of probiotics in aquaculture 248
4 Seaweed probiotic fermentation 249
5 Probiotic fortifies seaweed silage of *Eucheuma denticulatum* Doty 250
6 Chemical characteristics of seaweed silage 253

7 Seaweed silage as rotifer feed 253
8 Seaweed silage feed formulation 254
9 Conclusion 256
References 257

17 Secondary Metabolites From Probiotic Metabolism

María Chávarri, Lucía Diez-Gutiérrez, Izaskun Marañón and Luis Javier R. Barron

1 Probiotics 259
2 Postbiotics 260
2.1 Postbiotic classification 261
3 Conditions of probiotics to produce postbiotics 264
3.1 Culture media composition 264
3.2 Cultivation parameters 265
4 Human health benefits of probiotics and postbiotics 265
5 Application of probiotics and postbiotics for healthy food development 266
6 Conclusions 270
References 270

18 Bacteriocins Produced by Probiotic Microorganisms

Didem Deliorman Orhan

1 Introduction 277
2 Bacteriocins 278
3 Gram-negative bacteriocins 279
4 Gram-positive bacteriocins 280
4.1 Class I bacteriocins (modified peptides) 281
4.2 Class II bacteriocins (unmodified peptides) 281
4.3 Class III bacteriocins (large proteins) 281
5 The mechanism of antibacterial activity of bacteriocins 284
6 Applications of bacteriocins 285
6.1 Application in foods 285
6.2 Animal health 286
6.3 Human health 286
7 Conclusion 288
References 289

19 Probioactives: Bacteriocin and Exopolysaccharides

Marimuthu Anandharaj, Rizwana Parveen Rani and Manas Ranjan Swain

1 Introduction 293
2 Probioactive perception 293
3 Sources and strain specificity of probioactives 294
4 Bacteriocin from probiotic strains 294
5 Bacteriocin classification 295
6 Biochemical characterization of bacteriocin 295
7 Bacteriocin genetics and biosynthesis 295
8 Cytotoxicity effect of bacteriocin 296
9 Bacteriocin from *Lactobacillus* sp. 296
10 Gassericin from *Lactobacillus gasseri* 297
11 Bacteriocin from *Bacillus* species 297
12 Subtilosin A from *Bacillus subtilis* 297
13 Bacterial exopolysaccharides 298
14 EPS characteristics 298
15 EPS classification 298
16 Biosynthesis and genetics of EPS 300
17 EPS production 301
18 Physicochemical properties of EPS 301
19 Biological properties of bacterial EPS 302
20 Immunostimulatory activity 302
21 Antioxidant property 302
22 Anticancer effects 302
23 Cholesterol-lowering activity 303
24 Conclusion 303
References 303

20 Probiotics in Shrimp Aquaculture

S. Madhana, G. Kanimozhi and A. Panneerselvam

1 Introduction 309
2 Definition 309
3 Types of probiotics 309
4 Microorganisms of probiotic 310
5 Probiotics in aquaculture 310
5.1 Mechanisms of probiotics 310
5.2 Competitive exclusion 311
5.3 Nutrient and enzymatic contributions to digestion 311
6 Immune system promoters 311
7 Water quality improvement 311
8 Bioremediation 311
9 Materials and methods 312
10 Probiotic feed preparation 312
10.1 Tank culture experiments 312
11 Assessment of physicochemical parameters 313
12 Assessment of growth performance 313
13 Results and discussion 313
14 Determination of physicochemical parameters of probiotics supplemented shrimp aquaculture tank with various days of incubation 314
15 Biochemical analysis of *Litopenaeus vannamei* by the effect of potential bacteria in different intervals 317
16 Application of probiotics 320
17 Hydrogen peroxide (H_2O_2) 321
18 Influence of immune system 321

19 Effect of reproduction of aquatic species 322
20 Conclusions 322
References 323

21 Prospective Approaches of *Pseudonocardia alaniniphila* Hydrobionts for *Litopenaeus vannamei*

A.S. Shijila Rani, S. Babu, A. Anbukumaran, S. Veeramani, V. Ambikapathy, S. Gomathi and G. Senthilkumar

1 Introduction 327
1.1 Actinobacteria 327
1.2 Aquaculture 327
1.3 Shrimp diseases 328
1.4 Control of shrimp pathogen 328
1.5 Probiotics: definition and principles 328
2 Materials and methods 328
2.1 Study site 328
2.2 Sample collection 329
2.3 Analysis of physicochemical parameters in soil 329
2.4 Isolation and identification of Actinobacteria 329
2.5 Determination of antimicrobial activity 330
2.6 Molecular characterization of potential Actinobacteria 331
2.7 Experimental design and feeding management 331
2.8 Analysis of physicochemical parameters in water samples 332
2.9 Growth and development of *Litopenaeus vannamei* *332*
2.10 Biochemical analysis 332
2.11 Immunological studies 332
3 Results and discussion 332
3.1 Physicochemical properties of soil sample 333
3.2 Soil texture 333
3.3 pH 333
3.4 Electrical conductivity and cation exchange capacity 334
3.5 Organic carbon and organic matter 334
3.6 Macronutrients 334
3.7 Micronutrients 334
3.8 Isolation and identification of Actinobacteria from the mangrove soil samples 334
3.9 Screening of antimicrobial activity against human pathogen 337
3.10 Shrimp pathogen activity 337
3.11 Antibiotic sensitivity test 338
3.12 Molecular characterization of Actinobacterial strain 339
3.13 Experimental design 339
4 Conclusion 342
References 346

22 Probiotics as a Growth Promotant for Livestock and Poultry Production

Vinothini Gopal and Dharumadurai Dhanasekaran

1 Introduction 349
2 Exploitation of antibiotics in poultry production 349
2.1 Antibiotics and microbial resistance 350
2.2 Impact of antibiotic on the environment and consumer health 350
2.3 Alternatives to the usage of antibiotics 351
3 Probiotics: definition, concepts, and history 352
3.1 Microbes used as animal probiotics 352
4 Probiotics for poultry nutrition: a glance on the market 354
4.1 Selection of probiotics 355
5 Role of probiotics in poultry 356
6 Bacterial populations in GI tract of poultry 358
7 Application of actinobacteria as probiotics in livestock and poultry production 359
8 Conclusion 360
References 360

23 Small- and Large-Scale Production of Probiotic Foods, Probiotic Potential and Nutritional Benefits

Gazalla Akhtar, Naseer Ahmad Bhat, F.A. Masoodi and Adil Gani

1 Introduction 365
2 Role of probiotics in food fermentation 366
3 Production of probiotic foods 367
4 Major probiotic foods 367
4.1 Kimchi 367
4.2 Production of kimchi 368
4.3 Probiotic value of kimchi 368
4.4 Health benefits of kimchi 369
5 Tempeh 370
5.1 Manufacture of tempeh 371
5.2 Health benefits of tempeh 372
6 Kombucha 373
6.1 Probiotic potential of kombucha 374
6.2 Biochemical profile of kombucha tea 374
6.3 Health benefits of kombucha 375

7 Kefir 376
7.1 Probiotic value of kefir 376
7.2 Kefir production 377
7.3 Health benefits of kefir 377
8 Sauerkraut 379
8.1 Production of sauerkraut 379
8.2 Nutritional profile of sauerkraut 380
8.3 Health benefits of sauerkraut 380
9 Pickles 381
9.1 Production of pickles 382
9.2 Health benefits of pickle 382
10 Idli 383
10.1 Production of idli 383
10.2 Nutritional profile and health benefits 383
11 Miso 384
11.1 Production of miso 384
11.2 Nutritional profile and health benefits 384
12 Yoghurt 384
12.1 Production of yoghurt 385
12.2 Nutritional profile and health benefits 386
13 Dosa 386
13.1 Health benefits of dosa 387
14 Conclusion 387
References 387

24 Lactic Acid Bacteria in Fermented Food

Peng Chen

1 Introduction 397
2 The probiotics microorganism used in the fermented food 398
2.1 Acetobacter 398
2.2 Nonpathogenic *Corynebacterium* 398
2.3 Lactic acid bacteria 398
2.4 Yeast 399
2.5 Mold 404
2.6 Fermented vegetable food microorganism 405
2.7 Microorganism in fermented dairy products 410
2.8 Fermented meat and fish 410
2.9 Microorganism in fermented grain–based foods 410
2.10 Probiotics 410
2.11 Bacteria 411
3 Function and application of food microorganisms 413
3.1 Health function of probiotics microorganisms 413
3.2 Diseases caused by intestinal flora imbalance 414
4 Prospect 414
References 414

25 Commercially Available Probiotics and Prebiotics Used in Human and Animal Nutrition

Khalid Muzaffar, Romee Jan, Naseer Ahmad Bhat, Adil Gani and Mudasir Ahmed Shagoo

1 Introduction 417
2 Probiotic microorganisms used in human nutrition and their role 418
2.1 *Lactobacillus* species 418
2.2 *Bifidobacterium* species 421
2.3 Yeast 423
3 Probiotic microorganisms used in animal nutrition and their role 423
3.1 Lactobacillus 424
3.2 Bifidobacterium 424
3.3 Bacillus 425
3.4 Saccharomyces 425
4 Prebiotics used in human and animal nutrition 427
4.1 Fructans 427
4.2 Galacto-oligosaccharides (GOS) 428
4.3 Resistant starch (RS) and glucose-derived oligosaccharides 428
4.4 Miscellaneous oligosaccharides 429
4.5 Lactulose 429
4.6 Non-carbohydrates prebiotics 429
5 Concluding remarks 429
References 429

26 New Formulations and Products in Prebiotic Food

Mohamed Yousuff Mohamed Imran, Nazar Reehana, Gangatharan Muralitharan, Nooruddin Thajuddin and Dharumadurai Dhanasekaran

1 Introduction 437
2 Prebiotic dietary fiber sources 438
2.1 Beta-glucan 438
2.2 Inulin, oligofructose, and FOSs 438
2.3 GOSs 438
2.4 Isomaltooligosaccharides 438
2.5 Guar gum 438
2.6 Lactulose 438
2.7 RS and maltodextrin 438
2.8 Xylooligosaccharides and arabinooligosaccharides 439
3 Prebiotic production from food industry wastes and agricultural by-products 439
4 Development of prebiotic food products 439
5 Prebiotics safety 442
6 Food applications of prebiotics 443
7 Conclusion 444
References 445

27 Therapeutic Potential of Different Probiotic Foods

J. Anita Christie and S. Geet Andrea

1 Introduction 449
1.1 Probiotics 449
1.2 Prebiotics 449
1.3 Synbiotics 450
2 Criteria for the selection of probiotic food 450
3 Different types of probiotic food 450
3.1 Classification of fermented food is based on different substrates 450
3.2 Milk-based: Yogurt, cheese, and kefir 450
3.3 Cereal- and legume-based: idli, dosa, appam 457
3.4 Legume-based: tempeh, miso 459
3.5 Vegetable- and fruit-based: kombucha, pickles, kimchi, and sauerkraut 461
4 Conclusion 470
References 470

28 Main Technological Challenges Associated With the Incorporation of Probiotic Cultures into Foods

Marilena Marino, Nadia Innocente, Sofia Melchior, Sonia Calligaris and Michela Maifreni

1 Introduction to probiotic-containing functional foods 479
2 Probiotic foods on the market 480
3 Factors affecting probiotics' viability 481
3.1 Food matrix features 481
3.2 Processing conditions 483
3.3 Competition with starter cultures 483
3.4 Storage conditions 484
3.5 GIT transit 484
4 Intervention strategies 484
4.1 Strain selection and inoculation condition 484
4.2 Strain cultivation 486
4.3 Addition of protective ingredients 488
4.4 Encapsulation 489
5 Final considerations 490
References 491

29 Effective Probiotic Delivery: Current Trends and Future Perspectives

Mangala Lakshmi Ragavan and Nilanjana Das

1 Introduction 497
2 Probiotics 498
2.1 Definition 498
2.2 Health benefits 498
3 Selection of probiotic strains for technological performance 499
3.1 Oxygen tolerance 499
3.2 Acid tolerance 499
3.3 Bile acid tolerance 500
3.4 Temperature tolerance 500
4 Use of encapsulation technology for effective delivery of probiotics 500
4.1 Significance of cell survival during processing and storage 502
4.2 Significance of cell survival during GIT transit 502
4.3 Improvement of sensory characters and limitations 503
5 Microencapsulation of probiotics 503
5.1 Efficient matrices for microencapsulation of probiotics 503
5.2 Effective encapsulation methods 504
6 Nanoencapsulation of probiotics 506
6.1 Lipid-based nanocarriers 507
6.2 Nature-inspired nanocarriers 507
6.3 Special equipment-based nanocarriers 507
6.4 Biopolymer-based nanocarriers 508
7 Encapsulation of probiotics: insights into industrial applications 508
8 Conclusion and future perspectives 509
References 511

30 Industrial Requirements and Other Techno-functional Traits of Probiotics

Govindan Nadar Rajivgandhi, Vimala RTV, Govindan Ramachandran and Natesan Manoharan

1 Introduction 519
1.1 Characteristics of probiotics 520
2 Health benefits of probiotics 520
3 The techno-functional traits approaches of probiotics 522
3.1 Functional aspects of probiotics 523
3.2 Adhesion properties 523
3.3 Antagonistic properties 523
3.4 Immunomodulatory properties 524
3.5 Improved barrier function 524
3.6 Anti-inflammatory properties 524
3.7 Antimutagenic and anticarcinogenic properties 524
4 Industry-based probiotics application in various fields 525
4.1 Food applications of probiotics 525
4.2 Dairy-based probiotic foods 525
4.3 Fresh milk and fermented milks 525
4.4 Other dairy-based products 525
4.5 Fruit-based probiotic products 526

4.6 Cereal-based probiotic products 526
4.7 Meat-based probiotic foods 526
5 Agricultural applications of probiotics 526
6 Livestock applications of probiotics 527
7 Probiotics application challenges 527
7.1 Viability and survival 527
7.2 Sensory acceptance 528
8 The future of probiotics 528
9 Regulations and guidelines for probiotics 529
9.1 Safety aspects and harmful side effects of probiotics 529
10 Conclusion 530
References 530

Index 535

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Foreword



Probiotics, a global attracting term of the modern era, which denotes "for life" and it provides benefits to various biotic organisms. The main interest in probiotics is due to their vast health potential once it reaches the intestinal tract. Most of probiotic foods are classified under the category of functional foods and they hold special position in food market. In the past 2 decades, more efforts have been made by scientists and researchers that led to significant advancement of probiotics and its substantial health benefits to all the biotic organisms including humans. Due the great advances and breakthrough made in the field of probiotics in food and health, the topic deserve a review of recent progresses and developments to enable better understanding of future opportunities to maximize the benefits of probiotics.

Advances in Probiotics: Microorganisms in Food and Health edited by Dr. D. Dhanasekaran and Dr. A. Sankaranarayanan and published by **Elsevier is** a timely textbook that fill an urgent source of information on the topic. It comprises of more than 30 chapters, which deals with the beneficial effects of probiotics not only with human health but also in poultry, aquaculture, and industrial applications. The book contents expand the current knowledge and provide futuristic insights into next generation probiotics, probiotics role in cancer prevention and therapy, genetic modification and sequence analysis of probiotics, industrial need and other techno-functional traits, probiotics and *in silico* approach, probiotics in poultry, aquaculture, and their therapeutic potential.

I would like to congratulate the editors and all the chapter contributors for their voluminous efforts to bring the much needed book and for sharing their knowledge with the wider scientific community. I am sure that with this book, the readers are getting updated information regarding the probiotics and their health benefits.

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Preface

Probiotics, a demanding and an emerging field during the past 3 decades by continuous expanding its wings pointing to their applications in various fields including human health. As per WHO (2002) definition on probiotic stated that, "live strains of strictly selected microorganisms which, when administered in adequate amounts, confer a health benefit on the host." Scientific reports strongly emphasized the beneficial effects of probiotic foods in combating various life threatening diseases and health benefits.

In lieu of the above stand, we the editors are intended to publish a book on "**Advances in Probiotics: Microorganisms in Food and Health**" by inviting chapters from eminent researchers/expertise in this domain. The chapters are focused from basic aspects including overview of probiotic microorganisms and health benefits, nutritional properties of probiotic foods to role of probiotic in cancer prevention and therapy, genetic modification and sequence analysis, probiotic microbiome, probioactives, next generation probiotics, main technological challenges associated with the incorporation of probiotic cultures into foods, and industrial requirements and other techno-functional traits of probiotics. This book covers the application of probiotic in different fields including aquaculture, poultry science, animal nutrition, and industrial perspectives; and combating various human diseases and also sprawls about prebiotics and synbiotics.

This book will be benefit for the graduate, post graduate students, teachers, researchers, microbiologist, and other professionals, who are interested to fortify and expand their knowledge about probiotic microorganisms and their applications in the field of Food Science & Technology, Food & dairy Microbiology, Industrial Microbiology, Biotechnology, Biomedical Science, Plant & Animal Science, Agriculture Science, etc.

This book comprises a total of 31 chapters from multiple contributors around the world including Canada, China, Hungary, Italy, India, Malaysia, Poland, Spain**,** Slovenia, Turkey, The Republic of Korea, and United States of America. We are grateful to all the contributors and leading experts for the submission of their stimulating and inclusive chapters in the preparation of the edited volume to bring the book on Advances in Probiotics: Microorganisms in Food and Health. We offer our special thanks and appreciation to **Patricia Osborn**, Senior Acquisitions Editor, Elsevier Research Reference, Food and Marine Science and **Emerald Li,** Editorial Project Manager, Elsevier, 50 Hampshire Street, Cambridge MA, United States and their team for their constant encouragement and help in bringing out the volume in the current structure. We also record our gratitude to **Dr. Alaa El-Din Bekhit**, Department of Food Science, University of Otago, Dunedin, New Zealand for his support and help.

We are also indebted to **Elsevier**, **Bharathidasan University**, Tiruchirappalli, Tamil Nadu, India and **Uka Tarsadia University**, Surat, Gujarat, India for their concern, efforts, and support in the task of publishing this volume. We are confident and hopeful that the book provides an insight about various aspects of probiotics and render the updated information to the readers.

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Part I

Probiotics Microorganisms

1. Probiotic Microorganisms and Their Benefit to Human Health 3
2. Selection Criteria for Identifying Putative Probiont 23
3. Simulated Gastrointestinal System to Assess the Probiotic Properties Modified to Encapsulation of Probiotics and Their Survival Under Simulated Gastrointestinal System 37
4. Next-Generation Probiotics 45
5. Edible Mushrooms: A Promising Bioresource for Prebiotics 81

Chapter 1

Probiotic Microorganisms and Their Benefit to Human Health

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1 Introduction

The well-known definition of probiotics was originally accepted in 2001 at an expert consultation of international scientists working on behalf of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) (FAO/WHO, 2001). In 2002 an FAO/WHO working group produced guidelines to assist with the interpretation of the original document (FAO/WHO, 2002). For almost two decades the definition has been widely used in the scientific community. In 2014 the International Scientific Association for Probiotics and Prebiotics (ISAPP) published the most recent and widely accepted definition of probiotics as follows: "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill et al., 2014). There has been some confusion and misuse of the term "probiotic" since this definition came about. The definition implies a number of elements that make an organism probiotic: a living and well-defined organism(s), scientific and clinical evidence to support health benefits in certain populations at certain doses, and specific use of that organism(s) according to the scientific data in only certain hosts. It is with this definition in mind that this chapter has been written, probiotic organisms that have positive clinical evidence behind them to support health benefits in humans.

The most common probiotics are members of the genus *Lactobacillus* that has recently been reclassified into 25 genera (Zheng et al., 2020). Strains of the following probiotic species have remained in genus *Lactobacillus*: *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus gasseri*, *Lactobacillus johnsonii*, and others. In contrast the novel genera now include the following: *Lacticaseibacillus casei*, *Lacticaseibacillus rhamnosus*, *Lactiplantibacillus plantarum*, *Limosilactobacillus reuteri*, *Ligilactobacillus salivarius*, etc. Other common probiotics belong to genus *Bifidobacterium*, for example, strains of the following species and subspecies: *Bifidobacterium longum* subsp. *infantis*, *Bifidobacterium animalis* subsp. *lactis*, *B. longum*, *Bifidobacterium breve*, *Bifidobacterium bifidum*, and others. Also, strains from other bacterial species (e.g., *Pediococcus acidilactici*, *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides*, *Bacillus coagulans*, *Bacillus subtilis*, *Clostridium butyricum*, *Enterococcus faecium*, *Streptococcus thermophilus*, *Escherichia coli*, and others) and certain yeasts (e.g., *Saccharomyces boulardii*) qualify as probiotics (Fijan, 2014; Fijan et al., 2019a).

According to the ninth edition of the European Pharmacopoeia form 2019 (European Pharmacopoeia, 2019), "live biotherapeutic products" for human use are medicinal products containing live microorganisms that can be administered orally or vaginally in different pharmaceutical forms. New terms such as "paraprobiotic" or "postbiotic" have emerged to denote that nonviable microbial cells, microbial fractions, cell lysates, or microbial metabolites might also offer physiological benefits to the host by providing additional bioactivity (Pandey, Naik, & Vakil, 2015). On the other hand, "prebiotics" are substrates that are selectively used by host microorganisms conferring a health benefit (Gibson et al., 2017; Korcz, Kerényi, & Varga, 2018; Varga, Szigeti, & Gyenis, 2006) and "synbiotics" are dietary supplements or food ingredients that combine the effects of probiotics and prebiotics (Schrezenmeir & de Vrese, 2001; Varga & Andok, 2018; Varga, Szigeti, & Csengeri, 2003).

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00001-0
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With increasing knowledge about the essential role of gut microbiome in human health, gut microbiome is now considered an important ally, interacting with most human cells (Cani, 2018). The discovery of links, or axes, for instance, the "gut–brain" and "gut–brain–skin," has opened up new research dimensions. Besides mechanistic studies on fundamental topics (such as antimicrobial activity, competitive exclusion, immunomodulation, and strengthening of the intestinal epithelial barrier function), much research is focused on mechanisms of microbiome effects on the immune, the central nervous, and the endocrine systems (Clarke et al., 2014; Salem, Ramser, Isham, & Ghannoum, 2018; Zhou & Foster, 2015). Revolutionary discoveries about the importance of the human microbiome for human health have also accelerated further development of the probiotic sector. Historically, the probiotic sector led and fueled much microbiome research, and now the roles have reversed and these sister sciences are diverging into more independent, yet somewhat still connected fields. Scientific evidence of probiotic benefits on human health is continuously expanding, and there are enough data to justify investigation of probiotics for the treatment or prevention of several disorders from antibiotic and *Clostridioides difficile*–associated diarrhea, irritable bowel syndrome, and inflammatory bowel disease to anxiety, depression, and wound healing (Bagga et al., 2018; Lau & Chamberlain, 2016; Liu et al., 2019) (Fijan et al., 2019b). The phrase "when administered," in the definition of probiotics, can refer to the widespread application of probiotics in the gut as well as other sites (e.g., skin or vagina). Beneficial effects of probiotics have also been demonstrated for topical and per os use of probiotics in dental medicine, for female urogenital infections, and for the respiratory tract.

Taxonomy and nomenclature are important aspects of biological sciences, as they enable unambiguous communication about all living species. In contrast to higher life forms, bacteria have no comparable sexual reproduction, upon which speciation can be based. Therefore alternative criteria have to be used, which differ depending on the group of microorganisms. In the past, phenotypic criteria such as fermentation patterns, enzymatic profiles, and DNA–DNA hybridization were cornerstone techniques for speciation. Today, however, the wider availability of high-throughput sequencing technologies and the relatively small genome size of bacteria have allowed phenotypic testing to be replaced by genome sequencing as the main source of taxonomic information (Pot, Salvetti, Mattarelli, & Felis, 2019). The former *Lactobacillus* genus has been reclassified on the basis of both genotypic and phenotypic characteristics to achieve the most stable new groupings while balancing scientific accuracy and precision in the use of these novel techniques (Zheng et al., 2020). This chapter focuses on probiotics as microorganisms and their most common detection methods and major health benefits.

2 Most common detection methods and assays of probiotic microorganisms

The titles of in vitro studies, animal model studies, clinical studies, and reviews were screened using the ScienceDirect, PubMed, Web of Science databases, and the most commonly used assays for determining the characteristics of probiotics as well as phenotypic and genotypic methods for detecting and studying the characteristics of probiotic microorganisms that are noted.

2.1 In vitro assays

The most basic microbiological method for studying probiotics as microorganisms is the morphological assay. It usually involves fixation, cell preparation, such as simple or differential staining followed by imaging using optical microscopy (Reshes, Vanounou, Fishov, & Feingold, 2008). Phase contrast microscopy, fluorescent microscopy, and scanning microscopy are also common (Krasowska, Murzyn, Dyjankiewicz, Łukaszewicz, & Dziadkowiec, 2009). The vast majority of probiotics are Gram-positive bacteria and are therefore microscopically visible as violet rods (lactobacilli, bacilli, and clostridia) (Haghshenas et al., 2017), cocci (lactococci, enterococci, and streptococci), or V- or Y-shaped bifidobacteria (Jena, Choudhury, Puniya, & Tomar, 2017). In contrast, *E. coli* Nissle 1917 is a Gram-negative probiotic bacterium visible as pink rods under a microscope (Reshes et al., 2008). *S. boulardii* is a yeast species that belongs to eukaryotic microbes. Its cells are globose, ellipsoid, or elongated in shape (Krasowska et al., 2009). The absence or presence of bacterial and yeast spores can also be reported (Mattarelli et al., 2014).

Cultivation methods are based on the ability to culture probiotic organisms, that is, efficient multiplication of viable cells in nutrient broths and observing typical growth patterns resulting in displays of specific phenotypic traits in selective media. Lactic acid bacteria are usually grown in De Man, Rogosa, and Sharpe (MRS) broth (He et al., 2017). For other probiotic genera, various other nutrient broths are used, including brain heart infusion broth, tryptic soy broth, and M17 (Adouard et al., 2015). Selective solid media, depending on the probiotic genera, include MRS agar for lactobacilli (He et al., 2017), BSM agar for bifidobacteria (Jena et al., 2017), MacConkey agar for *E. coli* Nissle 1917 (Fijan, Šulc, & Steyer, 2018), bacillus agar for *B. subtilis* (Thankappan, Ramesh, Ramkumar, Natarajaseenivasan, & Anbarasu, 2015), kanamycin esculin azide agar for probiotic enterococci (Khalkhali & Mojgani, 2017), and various yeast extract agars for *S. boulardii* (Adouard et al., 2015).

The agar-well diffusion assay or the agar-spot assay (Dunne et al., 2001; Martín et al., 2019) is often used to determine the antagonistic activity of probiotics against potential pathogens, their sensitivity to antibiotics, or the absence of virulence genes (Thankappan et al., 2015). Coaggregation and coculturing assays are also applied to determine antimicrobial efficacy (Martín et al., 2019). In addition, various biofilm assays can be used to assess the real-life response of probiotic organisms to different environmental conditions as opposed to planktonic state assays that represent a more artificial, and nonadherent set of conditions (Barzegari et al., 2020; Salas-Jara, Ilabaca, Vega, & García, 2016).

Further phenotypic assays are utilized for the identification and characterization of probiotic species and strains. Bifidobacteria are characterized by their ability to ferment glucose via the bifidus shunt with acetic and lactic acids as main end products, and thus they are determined by the presence of fructose-6-phosphate phosphoketolase in cell free extracts (Mattarelli et al., 2014). Lactobacilli are characterized by the fermentation of glucose via three different pathways. Obligate homofermentative lactobacilli ferment hexoses almost exclusively to lactic acid, by the Embden–Meyerhof–Parnas (EMP) pathway, whereas pentoses and gluconate are not fermented, as they lack phosphoketolase. Facultative heterofermentative lactobacilli degrade hexoses to lactic acid by the EMP pathway and are also able to degrade pentoses, and often gluconate, as they possess both aldolase and phosphoketolase. Obligate heterofermentative lactobacilli degrade hexoses by the phosphogluconate pathway, producing lactate, ethanol or acetic acid, and carbon dioxide; moreover, pentoses are also fermented via this pathway (Mattarelli et al., 2014; Hammes & Vogel, 1995).

Biochemical phenotypic differentiation of probiotic bacteria can include the detection of acid production, such as production of short-chain fatty acids and other acids (i.e., lactic, acetic, and propionic acids) and the detection of acetate, butyrate, propionate, ethanol, carbon dioxide, indole, hydrogen sulfate, hydrogen peroxide, and urease pyrrolidonyl arylamidase formation. Deamination of arginine, nitrate, and nitrite reduction, hydrolysis of gelatin and of hippurate, bile-esculin tolerance test, acetyl methyl carbinol (a.k.a. Voges–Proskauer) test, hemolysis test, tellurite tolerance, and pyruvate utilization can also be evaluated. Miniaturized test combinations and test kits, most of which allow an indicator-based determination of sugar utilization or show reactions on the basis of their specific microbial enzymes, can also be utilized in the biochemical phenotypic differentiation of probiotic bacteria (Mattarelli et al., 2014; Martín et al., 2019; Nagpal et al., 2018).

Western blotting analysis, ELISA tests, and immunofluorescence assays are used to determine the presence or production of proteins, interleukins, and immunoglobulins by probiotic organisms (He et al., 2017; Shi et al., 2014). Enzymatic kits are also useful for determining the presence of bacteriocins, such as nisin A, mutacin B, bovamine, mersacidin, lacticin B, bacillocin, butyricin, salivaricin, pediocin, enterocin, leucocin A, lactococcin G, lactacin B, leuconocin S, lactocin 27, colibactin, microcin, and others (Massip et al., 2019; Gillor, Etzion, & Riley, 2008). Niche-specific assays can also be utilized to investigate particular properties relevant to that probiotic site of administration and/or action. The important traits of orally applied probiotics targeting the gastrointestinal tract include adhesion to intestinal epithelial cells, commonly conducted on Caco-2 cells (He et al., 2017; Martín et al., 2019), resistance to gastric acidity (Dunne et al., 2001; Thankappan et al., 2015), bile acid resistance (Dunne et al., 2001; Thankappan et al., 2015), tolerance to low pH (Thankappan et al., 2015), and capability to autoaggregate (Thankappan et al., 2015). In vitro exposure of probiotics to gastrointestinal-like conditions or simulators of gastrointestinal digestion can also be performed (Dunne et al., 2001; Martín et al., 2019). Probiotics targeting the upper respiratory tract would require assays to investigate direct antiviral activity and production of antiviral agent, stimulation of the immune system, and other host intestinal mucosal effects (Al Kassaa, Hober, Hamze, Chihib, & Drider, 2014; Wan, Chen, Shah, & El-Nezami, 2016), whereas vaginally applied probiotics would need to demonstrate strong lactic acid production, stimulation of vaginal mucosal immune factors, adhesion to and coaggregation with pathogenic organisms and within biofilms, and production of antibacterial compounds (Younes et al., 2018).

Molecular methods have greatly advanced over the past decade and include PCR and/or real-time PCR for multiplying genus-specific (Dubernet, Desmasures, & Guéguen, 2002; Bernhard & Field, 2000), species-specific (Sul, Kim, Kim, & Kim, 2007; Walter et al., 2000), and even strain-specific (Ahlroos & Tynkkynen, 2009) segments of probiotic DNA or whole-genome sequencing to distinguish species and strains based on 16S ribosomal RNA gene sequencing, multilocus sequence typing, random amplification of polymorphic DNA, and others (Mattarelli et al., 2014). MALDI–TOF (Martín et al., 2019) and DNA–DNA hybridization can also be used for species identification (De Andrés et al., 2018; Zheng et al., 2020; Mattarelli et al., 2014).

In the United States, probiotic strains are "generally recognized as safe." The qualified presumption of safety list also contains microorganisms as biological agents with safety risk assessments (Herman et al., 2019). However, the FAO and WHO have also recommended that probiotic strains be characterized at least with a series of in vitro tests, including antibiotic resistance patterns, metabolic activities, toxin production, and hemolytic activity. In vitro tests, such as bile salts resistance, can correlate with gastric survival in vivo, and spermicide resistance can help vaginal probiotics survive in users of these products. However, these two tests are exceptions and in vivo studies and/or clinical trials with beneficial

outcomes are necessary before a microorganism is labeled as probiotic (Reid, Jass, Sebulsky, & McCormick, 2003; FAO/WHO, 2001, 2002).

2.2 In vivo animal assays

All the aforementioned in vitro tests are very important to establish basic characteristics; however, the expression of such factors in vivo and verification that they comprise key mechanisms of action is needed before they can adequately predict the function of probiotic microorganisms in the human body. According to FAO and WHO documents, it is also recommended that probiotic strains be characterized for infectivity in immunocompromised animal models (Reid et al., 2003). Several in vivo animal studies of the efficacy of probiotics have been published using mice, rats, pigs, and other animal models. However, reliance on in vitro data or animal models alone is not sufficient because these data do not necessarily correlate or translate directly to clinical evidence presented in human studies and, in addition, the use of animal model studies is being restricted in the European Union (Fijan et al., 2019a; Dunne et al., 2001; Massip et al., 2019; Cencic, 2012).

2.3 Clinical studies

The most important assay of probiotics is the study of their health benefits, which is most efficiently conducted via double blind, randomized, placebo-controlled clinical studies (phase 2 clinical trials) with appropriate design, sample size, and primary outcome(s) to determine if a probiotic strain or probiotic product is efficacious. The outcome(s) should be a statistically and biologically significant improvement in condition, symptoms, signs, well-being or quality of life, reduced risk of disease or longer time period to the next occurrence, or faster recovery from illness. Phase 3 clinical trials, usually conducted with live therapeutic agents, compare the efficacy of using probiotics with standard treatment for a specific condition. The FAO and WHO have also recommended that side effects in humans and adverse incidents in consumers are reported (Reid et al., 2003; FAO/WHO, 2001, 2002).

3 Probiotic microorganisms and their recently reported health effects

The PubMed database was searched using the name of each individual probiotic species and the keyword "probiotics," limiting the hits to the most recent clinical studies, that is, those published between 2018 and 2020. Only full papers that included strain identification and studies that resulted in a reduction of symptoms or reduction of duration or prevention of a condition were included. Several studies with positive health benefits that used multispecies probiotics and prebiotic supplements were also included.

3.1 Probiotics, the genus *Lactobacillus*, and novel lactobacilli genera

Lactobacilli are widely used in industrial fermentations, with some species having a long history of safe and legal use in foods (Pot et al., 2019). In humans, lactobacilli are normally present in the vagina and in the gastrointestinal tract and, together with bifidobacteria, they are among the first bacteria to colonize the infant gut after delivery (Milani et al., 2017; Fijan, 2014). Their cells are Gram-positive, catalase-negative, nonendospore-forming rods or coccobacilli, which can also be motile when grown without heme in the medium. Lactobacilli are generally oxygen tolerant, aciduric or acidophilic, and obligately saccharoclastic with at least 50% of the carbohydrate end products being lactate and other fermentation products consisting of acetate, ethanol, CO_2, formate, and succinate. Several fermentation types can be recognized: obligately homofermentative, facultatively heterofermentative, and obligately heterofermentative metabolisms based on the type of sugars fermented (hexoses and pentoses) and fermentation products formed (Mattarelli et al., 2014; Hammes & Vogel, 1995). There are more than 200 published species within the *Lactobacillus* genus complex, the majority of which have sequenced type strain genomes available (Wittouck, Wuyts, Meehan, van Noort, & Lebeer, 2019); many of its species and strains are well-known probiotics. A recent whole-genome sequence analysis has shown that this genus is very heterogeneous, and that the genus has a spread that largely exceeds the normal spread of a genus. These findings have been confirmed by other studies showing that a formal split of the genus was unavoidable (Pot et al., 2014, 2019; Salvetti, Harris, Felis, & O'Toole, 2018; Sun et al., 2015).

Therefore according to the International Committee on Systematics of Prokaryotes, the genus *Lactobacillus* has been divided into new genera according to data based on genome and phenotypic comparisons. The generic term "lactobacilli" will be used to designate all bacteria classified as *Lactobacillaceae* until April 15, 2020; from that date onward the newly published names are the correct and official scientific genera to be used instead of the former genus *Lactobacillus*. A thorough discussion on the new names and their etymology has been carried out and appropriate names for the new groups have been found and accepted. The reclassification has been approved as follows: "the genus *Lactobacillus* is reclassified into 25 genera, including the emended genus *Lactobacillus*, which includes host-adapted organisms that have been referred to as the *L. delbrueckii* group, *Paralactobacillus* and 23 novel genera: *Holzapfelia*, *Amylolactobacillus*, *Bombilactobacillus*, *Companilactobacillus*, *Lapidilactobacillus*, *Agrilactobacillus*, *Schleiferilactobacillus*, *Loigolactobacillus*, *Lacticaseibacillus*, *Latilactobacillus*, *Dellaglioa*, *Liquorilactobacillus*, *Ligilactobacillus*, *Lactiplantibacillus*, *Furfurilactobacillus*, *Paucilactobacillus*, *Limosilactobacillus*, *Fructilactobacillus*, *Acetilactobacillus*, *Apilactobacillus*, *Levilactobacillus*, *Secundilactobacillus*, and *Lentilactobacillus*" (Mattarelli, Felis, Pot, Holzapfel, & Franz, 2020; Zheng et al., 2020). There are some very handy websites to compare old and new names as follows: http://lactobacillus.ualberta.ca/, http://lactotax.embl.de/wuyts/lactotax/, http://lactobacillus.uantwerpen.be/

3.1.1 Positive health effects of probiotic strains in the emended genus Lactobacillus reported between 2018 and 2020

As was previously mentioned, certain lactobacilli have remained in the reclassified genus *Lactobacillus*, including *L. acidophilus*, the *L. delbrueckii* group, *L. gasseri*, *Lactobacillus helveticus*, *Lactobacillus jensenii*, *L. johnsonii*, and others (Zheng et al., 2020). The results of clinical trials published from January 2018 through April 2020, and included in the PubMed database, were screened and noted later if any health effects due to supplementation with probiotics from the emended genus *Lactobacillus* were reported.

The main health benefits of these studies are reported. Supplementation with ***L. acidophilus*** **LA-5**, ***L. delbrueckii*** **subsp.** ***bulgaricus*** **LBY-27**, and other probiotics improved several inflammation and oxidative stress biomarkers in women with gestational diabetes (Hajifaraji et al., 2018), while supplementation with ***L. acidophilus*** **LA-5** and *B. animalis* subsp. *lactis* BB-12 reduced the incidence and severity of acute radiation-induced diarrhea in cervical cancer patients (Linn, Thu, & Win, 2019). Combined supplementation with ***L. acidophilus*** **GLA-14**, *L. rhamnosus* HN001, and lactoferrin reduced symptoms and recurrences of recurrent vaginal candidiasis (Russo, Superti, Karadja, & De Seta, 2019). ***L. acidophilus*** **CL1285** together with *L. casei* LBC80R and *L. rhamnosus* CLR2 improved symptoms of irritable bowel syndrome (Preston et al., 2018). ***L. acidophilus*** **BCMC 12130**, ***L. lactis*** **BCMC 12451**, and four other probiotic strains reduced proinflammatory cytokines in postsurgical colorectal cancer patients (Zaharuddin, Mokhtar, Muhammad Nawawi, & Raja Ali, 2019). ***L. acidophilus*** **CUL60** and **CUL21** and other probiotics administered before marathon race reduced gastrointestinal symptoms during race (Pugh et al., 2019). Supplementation with ***L. acidophilus*** **IBRC-M 10785** and two other probiotic strains had beneficial effects on serum high-sensitivity C-reactive protein, plasma nitric oxide, and malondialdehyde levels among people with overweight, diabetes, and coronary heart disease (Farrokhian et al., 2019), while administration of ***L. acidophilus*** **IBRC-M10785**, two other probiotic species, and inulin resulted in beneficial effects on pregnancy outcome among gestational diabetic women (Karamali et al., 2018). Salivary *Streptococcus mutans* count of female students was decreased by consumption of yoghurt containing ***L. acidophilus*** **ATCC 4356** and *B. bifidum* ATCC 29521 (Ghasemi, Mazaheri, & Tahmourespour, 2017). ***L. bulgaricus*** **OLL1073R-1** administration resulted in higher IFN-γ production in female health-care workers (Kinoshita et al., 2019) and increased the production of influenza A virus subtype of H3N2-bound salivary IgA in elderly residents of nursing homes (Yamamoto et al., 2019). Long-term supplementation with ***L. gasseri*** **CP2305** may improve the mental state, sleep quality, and gut microbiota of healthy adults under stressful conditions (Nishida, Sawada, Kuwano, Tanaka, & Rokutan, 2019). ***L. gasseri*** **BRN17** ingestion may contribute to reduced visceral fat mass in obese adults (Kim, Yun, Kim, Kwon, & Cho, 2018), while ***L gasseri*** **PA-3** was shown to improve serum uric acid levels in patients with hyperuricemia and/or gout (Yamanaka, Taniguchi, Tsuboi, Kano, & Asami, 2019). Yoghurt containing ***L. gasseri*** **DSM 22583**, ***L. jensenii*** **DSM 22567**, and *L. rhamnosus* DSM 22560 improved the recovery rate and symptoms of bacterial vaginosis in women (Laue et al., 2018). Supplementation with ***L. helveticus*** **R0052**, *B. longum* subsp. *infantis* R0033, and *B. bifidum* R0071 modulated certain immune factors (De Andrés et al., 2018).

3.1.2 Positive health effects of probiotic strains in the lactobacilli group other than the emended genus Lactobacillus reported between 2018 and 2020

As shown in Tables 1.1–1.3, the newly established lactobacilli genera with probiotic strains include *Lacticaseibacillus*, *Lactiplantibacillus*, *Ligilactobacillus*, *Limosilactobacillus*, and others (Zheng et al., 2020). The *Lacticaseibacillus* genus contains *Lacticaseibacillus casei* (previously known as *L. casei*), *Lacticaseibacillus paracasei* (formerly known as *L. paracasei*), *Lacticaseibacillus rhamnosus* (previously known as *L. rhamnosus*), and others (Table 1.1).

Supplementation with ***L. casei* Shirota**, *B. breve* Yakult, and galactooligosaccharides modulated the gut microbiota and reduced the incidence of enteritis and ventilator-associated pneumonia in patients with sepsis (Shimizu et al., 2018). ***L. casei* Shirota** was shown to reduce salivary *S. mutans* counts and plaque index scores in children as effectively as did fluoride mouthwash (Patil, Dastoor, & Unde, 2019), modulate the systemic and airways immune response post-marathon (Vaisberg et al., 2019), relieve pain intensity after single rib fracture (Lei et al., 2018), and prevent antibiotic-associated diarrhea and *C. difficile* infections in the intensive care unit (Alberda, Marcushamer, Hewer, Journault, & Kutsogiannis, 2018). ***L. casei* var. *rhamnosus*** reduced clinical severity in intestinal inflammatory reaction in children during acute diarrhea (Lai, Chiu, Kong, Chang, & Chen, 2019). Combined supplementation with ***L. casei* LBC80R**, *L. acidophilus* CL1285, and ***L. rhamnosus* CLR2** improved symptoms of irritable bowel syndrome (Preston et al., 2018). ***L. casei* BCMC 12313** and five other probiotic species reduced proinflammatory cytokines in postsurgical colorectal cancer patients (Zaharuddin et al., 2019). ***L. casei* CECT 9104** and two other probiotic species reduced the use of topical steroids in children with

TABLE 1.1 Overview of recent studies of the health effects of supplementation with probiotics in the genus *Lacticaseibacillus*.

Species with mentioned probiotic strain	Clinical studies published between January 2018 and April 2020		
	Number of studies found	Studies with reported probiotic strains and reported health benefits	
Lacticaseibacillus casei	12	11	Shimizu et al. (2018), Patil et al. (2019), Vaisberg et al. (2019), Lei et al. (2018), Alberda et al. (2018), Lai et al. (2019), Preston et al. (2018), Zaharuddin et al. (2019), Navarro-Lopez et al. (2018), Farrokhian et al. (2019), Karamali et al. (2018)
Lacticaseibacillus paracasei	9	4	Håkansson et al. (2019), Yoon et al. (2019), Huang et al. (2018a), Pahumunto et al. (2018)
Lacticaseibacillus rhamnosus	46	20	Okesene-Gafa et al. (2019), Dickerson et al. (2018), Alanzi et al. (2018), Schmidt et al. (2019), Durack et al. (2018), Wickens et al. (2018), Arnbjerg et al. (2018), Korpela et al. (2018), Lee et al. (2019), Ou et al. (2019), McMillan et al. (2018), Eggers et al. (2018), Crane et al. (2018), Vitellio et al. (2019), Russo et al. (2019), Gerasimov et al. (2018), Oda et al. (2019), Preston et al. (2018), Mantaring et al. (2018), Palma et al. (2018)

TABLE 1.2 Overview of recent studies of the health effects of supplementation with probiotics in the genera *Lactiplantibacillus* and *Ligilactobacillus*.

Species with mentioned probiotic strain	Clinical studies published between January 2018 and April 2020		
	Number of studies found	Studies with reported probiotic strains and reported health benefits	
Lactiplantibacillus plantarum	19	11	Håkansson et al. (2019), Liu et al. (2019), Chong et al. (2019), Hwang et al. (2019), Rudzki et al. (2019), Malik et al. (2018), Vladareanu et al. (2018), Culpepper et al. (2019), Kusumo et al. (2019), Huang et al. (2018b), Yoon et al. (2018)
Ligilactobacillus salivarius	4	2	Martín et al. (2019), Cárdenas et al. (2018)

TABLE 1.3 Overview of recent studies of the health effects of supplementation with probiotics in the genus *Limosilactobacillus*.

Species with mentioned probiotic strain	Clinical studies published between January 2018 and April 2020		
	Number of studies found	Studies with reported probiotic strains and reported health benefits	
Limosilactobacillus fermentum	2	1	Huang et al. (2018a)
Limosilactobacillus reuteri	30	14	Poonyam et al. (2019), Dore et al. (2019), Riezzo et al. (2019), Ou et al. (2019), McMillan et al. (2018), Gerasimov et al. (2018), Savino et al. (2018), Maragkoudaki et al. (2018), Alamoudi et al. (2018), Twetman et al. (2018), Galofré et al. (2018), Tenorio-Jiménez et al. (2019), Hsieh et al. (2018), Nilsson et al. (2018)

moderate atopic dermatitis (Navarro-Lopez et al., 2018). Administration of ***L. casei*** **IBRC-M 10783** and two other probiotic strains had beneficial effects on serum high-sensitivity C-reactive protein, plasma nitric oxide, and malondialdehyde levels among people with overweight, diabetes, and coronary heart disease (Farrokhian et al., 2019). ***L. casei*** **IBRC 10783**, two other probiotic species, and inulin beneficially influenced pregnancy outcome in gestational diabetic women (Karamali et al., 2018). Supplementation with ***L. paracasei*** **8700:2** and *L. plantarum* HEAL9 modulated the peripheral immune response in children with celiac disease autoimmunity (Håkansson et al., 2019). Supplementation with ***L. paracasei*** **HP7** and licorice reduced *Helicobacter pylori* density and improved histologic inflammation (Yoon et al., 2019). ***L. paracasei*** **GMNL-133** and *Limosilactobacillus fermentum* GM-090 aided clinical improvement in children with asthma (Huang, Chie, & Wang, 2018a). Supplementation with ***L. paracasei*** **SD-1** reduced *S. mutans* counts and delayed new caries development in young children (Pahumunto et al., 2018).

Supplementation with ***L. rhamnosus*** **GG** and *Bifidobacterium lactis* BB12 resulted in a modest reduction in total weight gain of pregnant women (Okesene-Gafa et al., 2019). The same combination of probiotics has also shown beneficial effects in various studies by (1) lowering the rate of rehospitalization in patients who have recently been discharged following hospitalization for mania (Dickerson et al., 2018), (2) promoting oral health in adolescents as a simple adjunct to standard oral care (Alanzi et al., 2018), (3) significantly lowering the incidence of eczema in late infancy (Schmidt et al., 2019), (4) improving microbiota development of infants with high risk for asthma at 6 months (Durack et al., 2018), (5) positively influencing outcomes across a spectrum of allergies in the first decade of life (Wickens et al., 2018), and (6) decreasing intestinal inflammation and the abundance of *Enterobacteriaceae* in HIV-infected individuals (Arnbjerg et al., 2018). ***L. rhamnosus*** **GG**, ***L. rhamnosus*** **Lc705**, *B. breve* Bb99, and *Propionibacterium freudenreichii* subsp. *shermanii* JS given to breastfeeding mothers and infants corrected undesired changes in microbiota composition due to antibiotic treatments or caesarean birth (Korpela et al., 2018). ***L. rhamnosus*** **SP1** supplementation significantly reduced the severity of *Candida*-associated denture stomatitis in institutionalized elderly patients (Lee, Vergara, & Lozano, 2019). Supplementation with ***L. rhamnosus*** **GR-1** and *L. reuteri* RC-14 may decrease the rates of mildly abnormal cervical smears (Ou et al., 2019). ***L. rhamnosus*** **GR-1** and *L. reuteri* RC-14 reduced the likelihood of preterm birth in pregnant women (McMillan et al., 2018). ***L. rhamnosus*** **HN001** administration reduced odds of carriage of *Staphylococcus aureus* in the gastrointestinal tract in veterans (Eggers et al., 2018) and also appeared to reduce eczema and early atopic sensation to food antigens during maternal supplementation (Crane et al., 2018). Combined supplementation with ***L. rhamnosus*** **HN001**, *B. longum* BB536, and vitamin B_6 significantly alleviated bloating. In addition, other symptoms and gut dysbiosis were also alleviated in lactose intolerant patients (Vitellio et al., 2019), while supplementation with ***L. rhamnosus*** **HN001**, *L. acidophilus* GLA-14, and lactoferrin reduced symptoms and recurrences of recurrent vaginal candidiasis (Russo et al., 2019). ***L. rhamnosus*** **19070-2** and *L. reuteri* 12246 reduced cry and fuss time in exclusively breastfed infants with colic (Gerasimov et al., 2018). Supplementation with ***L. rhamnosus*** **L8020** decreased periodontal disease in individuals with intellectual disability (Oda et al., 2019). ***L. rhamnosus*** **CLR2**, *L. acidophilus* CL1285, and *L. casei* LBC80R improved symptoms of irritable bowel syndrome (Preston et al., 2018). ***L. rhamnosus*** **CGMCC 1.3724** and *B. lactis* CNCC I-3446 showed beneficial effects of maternal supplementation on infant weight and length gains at 12 months (Mantaring et al., 2018). ***L. rhamnosus*** **BMX 54** resulted in restored eubiosis after HPV infection (Palma et al., 2018).

The newly established *Lactiplantibacillus* genus contains *Lactiplantibacillus plantarum* (previously known as *L. plantarum*), and the new *Ligilactobacillus* genus includes *Ligilactibacillus salivarius* (formerly known as *L. salivarius*) and other species (Table 1.2).

Supplementation with ***L. plantarum* HEAL9** and *L. paracasei* 8700:2 modulated the peripheral immune response in children with celiac disease autoimmunity (Håkansson et al., 2019). Supplementation with ***L. plantarum* PS128** significantly improved the total Swanson, Nolan, and Pelham-IV-Taiwan version score for boys with autism spectrum disorder (Liu et al., 2019), while supplementation with ***L. plantarum* DR7** alleviated stress and anxiety in adults (Chong et al., 2019). Soybeans fermented with ***L. plantarum* C29** enhanced cognitive function in individuals with mild cognitive impairment (Hwang et al., 2019). Similarly, supplementation with ***L. plantarum* 299v** improved cognitive functions in patients with major depression (Rudzki et al., 2019) and vascular endothelial function in men with stable coronary artery disease (Malik et al., 2018). ***L. plantarum* P17630** administration improved the vaginal colonization of women with recurrent vulvovaginal candidiasis (Vladareanu et al., 2018). A formulation of ***L. plantarum* HA-119**, *B. animalis* subsp. *lactis* B94, and *B. subtilis* R0179 resulted in different effects on plasma bile acid profiles in healthy obese adults (Culpepper et al., 2019). ***L. plantarum* IS-10506** increased fecal secretory immunoglobulin A in children younger than 2 years (Kusumo, Bela, Wibowo, Munasir, & Surono, 2019). ***L. plantarum* TWK10** improved endurance performance in adults (Huang et al., 2018b). ***L. plantarum* LRCC5193** and *S. thermophilus* MG510 significantly ameliorated stool consistency in patients with chronic constipation (Yoon et al., 2018). ***L. salivarius*** CECT 9145 administration reduced the number of *Streptococcus agalactiae*–positive women during pregnancy, thereby reducing the number of women receiving intrapartum antibiotic prophylaxis (Martín et al., 2019). As a result of supplementation with ***L. salivarius* PS7**, a reduction in the number of recurrent acute otitis media was reported in children (Cárdenas et al., 2018).

The new *Limosilactobacillus* genus contains *Limosilactobacillus fermentum* (previously known as *L. fermentum*) and *Limosilactobacillus reuteri* (formerly known as *L. reuteri*) (Table 1.3).

***L. fermentum* GM-090** and *L. paracasei* GMNL-133 proved to be beneficial to children with asthma (Huang et al., 2018a). ***L. reuteri* DSM 17938**, ***L. reuteri* ATCC PTA6475**, and a high-dose proton pump inhibitors (PPI)–bismuth-containing quadruple therapy resulted in an excellent cure rate for *H. pylori* infection as first-line treatment irrespective of CYP2C19 and antibiotic resistance pattern (Poonyam, Chotivitayatarakorn, & Vilaichone, 2019), while another study showed that administration with both strains could even be considered as a replacement when bismuth was contraindicated or unavailable (Dore et al., 2019). Long-term supplementation with ***L. reuteri* DSM 17938** also demonstrated an improvement in functional constipation (Riezzo et al., 2019). The combination of ***L. reuteri* RC-14** and *L. rhamnosus* GR-1 may decrease rates of mildly abnormal cervical smears (Ou et al., 2019) and may also reduce the likelihood of preterm birth in pregnant women (McMillan et al., 2018). In exclusively breastfed infants with colic, ***L. reuteri* 12246** and *L. rhamnosus* 19070-2 reduced cry and fuss time (Gerasimov et al., 2018). Similarly, a decrease in crying time was observed after supplementation with ***L. reuteri* DSM 17938** of infants with colic (Savino et al., 2018). Supplementation with the same strain and zinc also caused a reduction in severity of acute diarrhea in infants (Maragkoudaki et al., 2018). Investigations in oral health show further positive results: ***L. reuteri* DSM 17938** and ***L. reuteri* ATCC PTA 5289** significantly reduced caries-associated bacterial counts (Alamoudi, Almabadi, El Ashiry, & El Derwi, 2018), improved oral wound healing (Twetman, Keller, Lee, Yucel-Lindberg, & Lynge Pedersen, 2018), and improved clinical parameters of implants with mucositis or peri-implantitis (Galofré, Palao, Vicario, Nart, & Violant, 2018). Furthermore, ***L. reuteri* v3401** improved selected inflammatory parameters and modified the gastrointestinal microbiome (Tenorio-Jiménez et al., 2019). Consumption of ***L. reuteri* ADR-1** resulted in significant reductions in HbA1c and serum cholesterol of type 2 diabetes patients (Hsieh et al., 2018) and ***L. reuteri* ATCCPTA 6475** reduced bone loss in older women with low bone mineral density (Nilsson, Sundh, Bäckhed, & Lorentzon, 2018).

3.2 Probiotics and the genus *Bifidobacterium*

The genus *Bifidobacterium* includes various Gram-positive, nonmotile, anaerobic bacteria and contains many probiotic strains. They are endosymbiotic inhabitants of the gastrointestinal tract and vagina of mammals, including humans (Chen, Cai, & Feng, 2007; Fijan, 2014). The DNA G + C content of genus *Bifidobacterium* ranges from 50 to 65 mol%. Genera of the family *Bifidobacteriaceae* are characterized by the key catabolic action on fructose-6-phosphate by fructose-6-phosphate phosphoketolase and subsequent reactions via the pentose phosphate cycle. The bifidus shunt is the most important genus-specific characteristic. Acetic and lactic acids are formed at a molar ratio of 3 to 2. CO_2 is not normally produced, except during the degradation of gluconate. Small amounts of ethanol, formic acid, and succinic acid are also formed, whereas no butyric or propionic acids are produced (Biavati & Mattarelli, 2006; Mattarelli et al., 2014).

3.2.1 Positive health effects of probiotic strains in the genus Bifidobacterium reported between 2018 and 2020

Results of published clinical studies from the PubMed database between January 2018 and April 2020 were screened, and any positive health effects due to supplementation with probiotics from the genus *Bifidobacterium* were noted in the following sections and in Table 1.4.

Supplementation of ***B. animalis* subsp. *lactis* BB12**, and *L. rhamnosus* GG resulted in a modest reduction in total weight gain of pregnant women (Okesene-Gafa et al., 2019). The same combination of probiotics has also shown positive effects in several studies by decreasing the rate of rehospitalization in patients who have recently been discharged following hospitalization for mania (Dickerson et al., 2018), significantly reducing the incidence of eczema in late infancy (Schmidt et al., 2019), and promoting the oral health of adolescents as a simple adjunct to standard oral care (Alanzi et al., 2018). ***B. lactis* BB-12** and other probiotics have improved several inflammation and oxidative stress biomarkers in women with gestational diabetes (Hajifaraji et al., 2018), while supplementation with ***B. animalis* subsp. *lactis* BB-12**, and *L. acidophilus* LA-5 reduced the incidence and severity of acute radiation-induced diarrhea in cervical cancer patients (Linn et al., 2019). The abundance of gastrointestinal bifidobacteria in preterm infants was increased with administration of ***B. lactis* BB-12** and other probiotics, thus reducing the risk of necrotizing enterocolitis (Plummer et al., 2018). Hypocholesterolemic effects were observed due to supplementation with yogurt enriched with ***B. lactis* BB-12** and *L. lactis* 11/19-B1 (Nishiyama et al., 2018). Use of ***B. lactis* HN019** as an adjunct to scaling and root planing provided additional clinical, microbiological, and immunological benefits in the treatment of chronic periodontitis (Invernici et al., 2018). Synbiotic supplementation with ***B. lactis* BB-12** and prebiotics improved liver steatosis and liver enzyme concentrations in patients with nonalcoholic fatty liver disease (Bakhshimoghaddam, Shateri, Sina, Hashemian, & Alizadeh, 2018). A maternal nutritional supplement enriched with ***B. lactis* CNCC I-3446** and *L. rhamnosus* CGMCC 1.3724 given during late pregnancy and early lactation beneficially influenced infant weight and length gains at 12 months (Mantaring et al., 2018). ***B. animalis* subsp. *lactis* B94**, *L. plantarum* HA-119, and *B. subtilis* R0179 use resulted in different effects on plasma bile acid profiles in healthy obese adults (Culpepper et al., 2019). In marathon runners a multispecies probiotic product containing ***B. lactis* CUL34** and other probiotic strains consumed before a marathon race reduced gastrointestinal symptoms during the race (Pugh et al., 2019). ***B. animalis* subsp. *lactis* LKM512** and arginine administration significantly increased the reactive hyperemia index and other parameters, thus reducing the risk of atherosclerosis (Matsumoto, Kitada, & Naito, 2019). ***B. lactis* 420** consumption resulted in reduced plasma bile acids, glycocholic acid, and other acids of overweight adults (Hibberd et al., 2019). Finally,

TABLE 1.4 Overview of recent studies of the health effects of supplementation with probiotics in the genus *Bifidobacterium*.

Species with mentioned probiotic strain	Clinical studies published between January 2018 and April 2020		
	Number of studies found	Studies with reported probiotic strains and reported health benefits	
Bifidobacterium animalis subsp. *lactis*	27	16	Okesene-Gafa et al. (2019), Dickerson et al. (2018), Schmidt et al. (2019), Alanzi et al. (2018), Hajifaraji et al. (2018), Linn et al. (2019), Plummer et al. (2018), Nishiyama et al. (2018), Invernici et al. (2018), Bakhshimoghaddam et al. (2018), Mantaring et al. (2018), Culpepper et al. (2019), Pugh et al. (2019), Matsumoto et al. (2019), Hibberd et al. (2019), Navarro-Lopez et al. (2018)
Bifidobacterium bifidum	15	8	Pugh et al. (2019), De Andrés et al. (2018), Zaharuddin et al. (2019), Gomi et al. (2018), Karamali et al. (2018), Farrokhian et al. (2019), Bazanella et al. (2017), Ghasemi et al. (2017)
Bifidobacterium breve	9	7	Shimizu et al. (2018), Candy et al. (2018), Inoue et al. (2018), Korpela et al. (2018), Mortensen et al. (2019), Kobayashi et al. (2019), Bazanella et al. (2017)
Bifidobacterium longum	18	10	Plummer et al. (2018), De Andrés et al. (2018), Vitellio et al. (2019), Lau et al. (2018), Zaharuddin et al. (2019), Wang et al. (2019), de Cássia Stampini Oliveira Lopes et al. (2019), Navarro-Lopez et al. (2018), Inoue et al. (2018), Bazanella et al. (2017)

the administration of ***B. lactis*** **CECT 8145**, ***B. longum*** **CECT 7347**, and another probiotic species reduced topical steroid use in children with moderate atopic dermatitis (Navarro-Lopez et al., 2018).

B. bifidum **CUL20** used in combination with other probiotic strains before a marathon race reduced gastrointestinal symptoms during the race (Pugh et al., 2019). Together, ***B. bifidum*** **R0071**, *L. helveticus* R0052, and *Bifidobacterium infantis* R0033 modulated certain immune factors (De Andrés et al., 2018). ***B. bifidum*** **BCMC 02290**, ***B. infantis*** **BCMC 02129**, ***B. longum*** **BCMC 02120**, and three other probiotic strains reduced proinflammatory cytokines in postsurgical colorectal cancer patients (Zaharuddin et al., 2019). Consumption of milk fermented with ***B. bifidum*** **YIT 10347** resulted in significantly higher relief rates of overall gastrointestinal symptoms, upper gastrointestinal symptoms, flatus, and diarrhea in healthy adults (Gomi et al., 2018). Synbiotic supplementation with ***B. bifidum*** **IBRC-M10771**, two other probiotic strains, and inulin beneficially influenced pregnancy outcomes among gestational diabetic women (Karamali et al., 2018). Supplementation with the same strain, that is, ***B. bifidum*** **IBRC-M10771**, and two additional probiotic strains had positive effects on serum high-sensitivity C-reactive protein, plasma nitric oxide, and malondialdehyde levels in people with overweight, diabetes, and coronary heart disease (Farrokhian et al., 2019). Combined supplementation with ***B. bifidum*** **BF3**, ***B. longum*** **subsp.** ***infantis*** **BT1**, ***B. longum*** **BG7**, ***B. breve*** **BR3**, and two other probiotic strains decreased occurrence of *Bacteroides* and *Blautia* spp. associated with changes in lipids and unknown metabolites in month-old infants, though these profiles converged later in the study (Bazanella et al., 2017). Consumption of yoghurt containing ***B. bifidum*** **ATCC 29521** and *L. acidophilus* ATCC 4356 was shown to reduce salivary *S. mutans* counts in female students (Ghasemi et al., 2017). Synbiotic supplementation with ***B. breve*** **Yakult**, *L. casei* Shirota, and galactooligosaccharides reduced the incidence of enteritis and ventilator-associated pneumonia in patients with sepsis (Shimizu et al., 2018), while consumption of ***B. breve*** **Yakult** in combination with a prebiotic improved the median percentage of bifidobacterial and lowered the *Eubacterium rectale*/*Clostridium coccoides* ratio in the fecal microbiota of non-IgE-mediated allergic infants (Candy et al., 2018). In healthy elderly subjects, combined probiotic bifidobacteria (***B. breve*** **M-16V**, ***B. breve*** **B-3**, alongside other strains) and moderate resistance training improved mental condition, body weight, and bowel movement frequency (Inoue et al., 2018). ***B. breve*** **Bb99**, *L. rhamnosus* GG, *L. rhamnosus* Lc705, and *P. freudenreichii* subsp. *shermanii* JS given to breastfeeding mothers and infants showed the expected increase in bifidobacterial and reductions in *Proteobacteria* and *Clostridia* that are associated with antibiotic treatments or caesarean birth (Korpela et al., 2018). Consumption of ***B. breve*** **Bif195** safely reduced the risk of small intestinal enteropathy caused by acetylsalicylic acid (Mortensen et al., 2019), while ***B. breve*** **A1** improved the immediate memory of elderly subjects with memory complaints (Kobayashi, Kuhara, Oki, & Xiao, 2019). Combined supplementation with ***B. longum*** **subsp.** ***infantis*** **BT1**, ***B. longum*** **BG7**, ***B. breve*** **BR3**, and two other probiotic strains decreased occurrence of *Bacteroides* and *Blautia* spp. associated with changes in lipids and unknown metabolites in month-old infants, though these profiles converged later in the study (Bazanella et al., 2017).

Supplementation with ***B. longum*** **subsp.** ***infantis*** **BB-02** and other probiotic strains increased the abundance of bifidobacteria in the gut of preterm infants, thus reducing the risk of necrotizing enterocolitis (Plummer et al., 2018). Certain immune factors were shown to be modulated by the combination of ***B. longum*** **subsp.** ***infantis*** **R0033**, *L. helveticus* R0052, and *B. bifidum* R0071 (De Andrés et al., 2018). Supplementation with ***B. longum*** **subsp.** ***longum*** **BB536**, *L. rhamnosus* HN001, and vitamin B_6 enriched several genera involved in lactose digestion, including bifidobacteria in lactose intolerant patients (Vitellio et al., 2019), while supplementation with ***B. longum*** **subsp.** ***longum*** **BB536** provided protective effects against upper respiratory illnesses—including increasing the abundance of the genus *Faecalibacterium* that is associated with antiinflammatory and immunomodulatory properties—in preschool Malaysian children (Lau et al., 2018). The brain activity of healthy volunteers during social stress was modulated by consumption of ***B. longum*** **1714** (Wang et al., 2019), while synbiotic administration of ***B. longum*** **BL-G301** and a prebiotic reduced serum uremic toxins and urea in hemodialysis individuals (de Cássia Stampini Oliveira Lopes et al., 2019). Finally, in healthy elderly subjects, the combination of probiotic bifidobacteria (***B. longum*** **subsp.** ***longum*** **BB536**, ***B. longum*** **subsp.** ***infantis*** **M-63**, and others) and moderate resistance training was shown to improve mental condition, body weight, and bowel movement frequency (Inoue et al., 2018).

3.3 Genera of probiotic lactic acid bacteria other than lactobacilli

Probiotic organisms belonging to the genera *Enterococcus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus* are Gram-positive, coccoid, nonendospore-forming, facultative anaerobic lactic acid bacterial strains. Enterococci are catalase-negative, halophilic, active glucose fermenters that survive at temperatures of 5°C–65°C and are capable of growing at pH values ranging from 4.5 to 10; some species are carboxyophilic and resistant to bile (Mattarelli et al., 2014). Lactococci are commonly used in the dairy industry for manufacturing fermented foods (Molnár, Gyenis, & Varga, 2005). They are important in preventing the growth of spoilage bacteria in dairy products due to acidification. Pediococci homofermentatively produce lactic acid from glucose, without CO_2 formation, and grow at pH values between 5 and 9 (Mattarelli et al., 2014).

TABLE 1.5 Overview of recent studies of the beneficial health effects of supplementation with nonlactobacilli lactic acid bacteria.

Genus with mentioned probiotic strain	Clinical studies published between January 2018 and April 2020		
	Number of studies found	Studies with reported probiotic strains and reported health benefits	
Enterococcus	6	1	Sun et al. (2019)
Lactococcus	10	4	Nishiyama et al. (2018), Beltrán-Barrientos et al. (2018), Komano et al. (2018), Ozaki et al. (2018)
Leuconostoc	1	0	–
Streptococcus	7	3	Yoon et al. (2018), Hajifaraji et al. (2018), Plummer et al. (2018)
Pediococcus	2	0	–

3.3.1 Positive health effects of probiotic strains among nonlactobacilli lactic acid bacteria reported between 2018 and 2020

The results of clinical studies published from January 2018 through April 2020 and found in the PubMed database were screened and noted later, including in Table 1.5, if any health effects due to supplementation with probiotic lactic acid bacteria other than lactobacilli were observed.

Supplementation with ***E. faecium* R0026**, *B. subtilis* R0179, and esomeprazole had a beneficial effect on reflux esophagitis treatment, including longer relapse time and lower risk of relapse (Sun, Wang, Sun, Zhang, & Zhang, 2019). Hypocholesterolemic effects were observed due to the consumption of yogurt enriched with ***L. lactis* 11/19-B1** and *B. lactis* BB-12 (Nishiyama et al., 2018) and that of milk fermented with ***L. lactis* NRRL B-50571** (Beltrán-Barrientos et al., 2018). Supplementation with heat-killed ***L. lactis* JCM 5805** relieved symptoms of upper respiratory tract infections of male athletes (Komano et al., 2018). Fermented milk containing ***L. lactis* subsp. *cremoris* FC** was beneficial for improving defecation and fecal properties of healthy young Japanese women (Ozaki et al., 2018). Supplementation with ***S. thermophilus* MG510** and *L. plantarum* LRCC5193 significantly ameliorated stool consistency in patients with chronic constipation (Yoon et al., 2018). ***S. thermophilus* STY-31** in combination with other probiotic strains improved several inflammation and oxidative stress biomarkers in women with gestational diabetes (Hajifaraji et al., 2018). Combined supplementation with ***S. thermophilus* TH-4** and several probiotic bifidobacterial strains increased the abundance of *Bifidobacterium* spp. in the gut microbiota of preterm infants, thereby reducing the risk of necrotizing enterocolitis (Plummer et al., 2018). No health benefits for supplementation with *L. mesenteroides* or other *Leuconostoc* species or *P. acidilactici* or other pediococci have been reported in published clinical trials since 2018.

3.4 Genera of other probiotic bacteria and yeasts

The genera *Bacillus* and *Clostridium* include Gram-positive, spore-forming, rod-shaped bacteria. Although several species are virulent pathogens, there are also some probiotic strains used for human consumption and animal nutrition. Even the genus *Escherichia*, which belongs to the Gram-negative, rod-shaped, nonspore-forming family of *Enterobacteriaceae* and is mainly known for its highly virulent serotypes, has one probiotic strain (Fijan, 2014). The genus *Propionibacterium* contains Gram-positive, anaerobic, rod-shaped bacteria with a unique metabolism, by which they are capable of synthesizing propionic acid by using unusual transcarboxylase enzymes. In addition, *P. freudenreichii* (*Propionibacterium shermanii*) is also commercially important for the production of vitamin B_{12} (Roessner, Huang, Warren, Raux, & Scott, 2002). The genus *Saccharomyces*, which includes various yeast species widely used for making wine, beer, and bread, has some well-known probiotic strains as well (Capece et al., 2018; Fijan, 2014).

3.4.1 Positive health effects of other probiotic bacteria and yeasts reported between 2018 and 2020

The results of clinical trials published between January 2018 and April 2020, and available in the PubMed database, were screened and noted later, including in Table 1.6, if any health effects due to supplementation with probiotic fungi or bacteria other than lactic acid bacteria and bifidobacteria were reported.

Supplementation with ***B. subtilis* R0179**, *B. animalis* subsp. *lactis* B94, and *L. plantarum* HA-119 resulted in different effects on plasma bile acid profiles in healthy obese adults (Culpepper et al., 2019). Combined supplementation with

TABLE 1.6 Overview of recent studies of the health effects of supplementation with other probiotic bacteria and yeasts.

Genus with mentioned probiotic strain	Clinical studies published between January 2018 and April 2020		
	Number of studies found	Studies with reported probiotic strains and reported health benefits	
Bacillus	5	4	Culpepper et al. (2019), Sun et al. (2019), Soman and Swamy (2019), Hatanaka et al. (2018)
Clostridium	4	3	Miyaoka et al. (2018), Chen et al. (2018), Xia et al. (2018)
Escherichia	2	2	Manzhalii et al. (2016), Faghihi et al. (2015)
Propionibacterium	3	1	Korpela et al. (2018)
Saccharomyces	8	3	He et al. (2019), Seddik et al. (2019), Carstensen et al. (2018)

B. subtilis* R0179**, *E. faecium* R0026, and esomeprazole beneficially influenced reflux esophagitis treatment, including longer relapse time and lower risk of relapse (Sun et al., 2019). ***B. coagulans* SNZ 1969**, ***Bacillus clausii* SNZ 1971**, and ***B. subtilis* SNZ 1972** administration resulted in a significant improvement in several symptoms of gastrointestinal discomfort (Soman & Swamy, 2019). Ingestion of ***B. subtilis* C-3102** spores significantly decreased the Bristol Stool Scale score and stool frequency in healthy volunteers with loose stools (Hatanaka et al., 2018). Coadministration of ***C. butyricum* MIYAIRI 588** in combination with antidepressants showed effectiveness as adjunctive therapy for treatment-resistant major depressive disorder (Miyaoka et al., 2018). A bismuth-containing quadruple therapy coupled with ***C. butyricum* CBM 588** supplementation was associated with improved gastrointestinal symptoms and an increased *Bacteroidetes*-to-*Firmicutes* ratio in *H. pylori*–positive patients (Chen et al., 2018). Supplementation with probiotics containing ***C. butyricum* CGMCC0313-1** and *B. infantis* CGMCC0313-2 improved symptoms, providing a new adjuvant therapy for the management of minimal hepatic encephalopathy in patients with HBV-induced cirrhosis (Xia et al., 2018). Intestinal-borne facial dermatoses were significantly improved by oral application of ***E. coli* Nissle 1917** (Manzhalii, Hornuss, & Stremmel, 2016). Diarrhea-predominant patients with irritable bowel syndrome showed a positive response, in the form of improved sleep quality, to probiotic supplementation with ***E. coli* Nissle 1917** (Faghihi, Agah, Masoudi, Ghafoori, & Eshraghi, 2015). ***P. freudenreichii* subsp. *shermanii* JS**, *B. breve* Bb99, *L. rhamnosus* GG, and *L. rhamnosus* Lc705 given to breastfeeding mothers and infants corrected undesired changes in infant fecal microbiota composition resulting from antibiotic treatments or caesarean birth (Korpela et al., 2018). A bismuth-containing quadruple therapy combined with ***Saccharomyces cerevisiae* var. *boulardii supplementation was used for *H. pylori* eradication and it was found to reduce the overall incidence of adverse reactions (He et al., 2019). Similarly, application of ***S. boulardii* CNCM I-745** to sequential therapy improved *H. pylori* eradication rate and reduced the incidence of treatment-associated adverse events in Moroccan patients (Seddik et al., 2019). ***S. boulardii* DBVPG** was shown to be effective in preventing *C. difficile* infections in elderly patients treated with antibiotics at internal medicine departments with high infection rates (Carstensen et al., 2018).

3.5 Novel or next-generation probiotics

Novel microorganisms are either newly isolated genera and species from various natural sources or strains derived from existing bacteria (Brodmann et al., 2017). Recent examples of microbes calling for more detailed evaluation include *Bacteroides xylanisolvens*, *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Parabacteroides goldsteinii*, *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, *Prevotella copri*, *Christensenella minuta*, and fructophilic lactic acid bacteria (Brodmann et al., 2017). Traditional probiotics, for example, *Lactobacillus* spp. and *Bifidobacterium* spp., were mostly selected randomly or through gathering living experiences (Chang et al., 2019). While most of them show biological safety and some of them may show ameliorative effectiveness, however, the general effects and functions on amelioration of diseases are statistically marginal. On the other hand, the administration of traditional probiotics does not aim against specific diseases. Based on these situations, identification and characterization of novel and disease-specific next-generation probiotics are urgently needed (Chang et al., 2019; Bottacini, Van Sinderen, & Ventura, 2017). Although many commensal gut microbes can potentially provide health benefits, an ISAPP expert panel has proposed conducting a strain-by-strain assessment until a sufficient body of scientific evidence is amassed to grant probiotic status at the species level. For this reason, clinical studies using next-generation probiotics are essential to determine the probiotic properties of novel microbial strains (Brodmann et al., 2017; Hill et al., 2014).

4 Discussion and conclusions

Probiotic research has considerably advanced over the last decade, with techniques focusing more on molecular and sequencing methods than on phenotypic techniques for species identification in addition to burgeoning clinical data for new indications. The *Lactobacillus* genus was extremely diverse from genetic, metabolic, ecological, and functional perspectives, and, therefore, a stable reclassification was necessary to prevent future additional reclassifications. As a result, major changes in the taxonomy of lactobacilli have been approved by the scientific community (Zheng et al., 2020). New probiotic strains and species, alongside new indications for health benefits, are challenging the probiotic industry and perception.

It is well known that probiotic supplementation may influence microbiota composition, improve intestinal health, be beneficial for traveler's diarrhea, constipation, antibiotic- and *C. difficile*-associated diarrhea, and even modulate the gut-associated immune system (Bagga et al., 2018; Maldonado Galdeano, Cazorla, Lemme Dumit, Vélez, & Perdigón, 2019; Liu, Alookaran, & Rhoads, 2018; Lau & Chamberlain, 2016). However, clinical trials have recently focused on determining the influence of probiotic supplementation on mental conditions via the gut–brain axis as well as their efficiency against allergies, including eczema, vaginal and oral health, and even obesity. Also, the focus is being shifted to the use of novel strains, and even novel species, as probiotics. The application of probiotics in the prevention and management of different conditions is very promising. Unfortunately, several recent clinical trials have failed to report the specific strains used. Although a few core benefits can be ascribed to certain species, or even the general category of probiotics (Hill et al., 2014), the particular strains used in clinical trials should be reported in order to achieve reproducibility of results.

However, it remains a fact that no single probiotic is efficient for all conditions and all individuals, and certain evidence-based combinations are more efficient than others, notwithstanding the unresolved topics of single versus multiple strains products, matrix effects for probiotics, and understanding the functional health impacts of probiotics on the microbiotas (McFarland, 2020; Fijan, 2014). Continued research is needed on the health benefits of probiotic microorganisms, especially in the form of well-designed, randomized, double-blinded, and placebo-controlled human trials and additional in vitro studies to further understand probiotic mechanisms of action.

References

Adouard, N., Foligné, B., Dewulf, J., Bouix, M., Picque, D., & Bonnarme, P. (2015). In vitro characterization of the digestive stress response and immunomodulatory properties of microorganisms isolated from smear-ripened cheese. *International Journal of Food Microbiology*, *197*, 98–107. https://doi.org/10.1016/j.ijfoodmicro.2014.12.011.

Ahlroos, T., & Tynkkynen, S. (2009). Quantitative strain-specific detection of *Lactobacillus rhamnosus* GG in human faecal samples by real-time PCR. *Journal of Applied Microbiology*, *106*(2), 506–514. https://doi.org/10.1111/j.1365-2672.2008.04018.x.

Al Kassaa, I., Hober, D., Hamze, M., Chihib, N. E., & Drider, D. (2014). Antiviral potential of lactic acid bacteria and their bacteriocins. *Probiotics and Antimicrobial Proteins*, *6*, 177–185. https://doi.org/10.1007/s12602-014-9162-6.

Alamoudi, N. M., Almabadi, E. S., El Ashiry, E. A., & El Derwi, D. A. (2018). Effect of probiotic *Lactobacillus reuteri* on salivary cariogenic bacterial counts among groups of preschool children in Jeddah, Saudi Arabia: A randomized clinical trial. *Journal of Clinical Pediatric Dentistry*, *42*(5), 331–337. https://doi.org/10.17796/1053-4625-42.5.2.

Alanzi, A., Honkala, S., Honkala, E., Varghese, A., Tolvanen, M., & Söderling, E. (2018). Effect of *Lactobacillus rhamnosus* and *Bifidobacterium lactis* on gingival health, dental plaque, and periodontopathogens in adolescents: A randomised placebo-controlled clinical trial. *Beneficial Microbes*, 1–10. https://doi.org/10.3920/BM2017.0139.

Alberda, C., Marcushamer, S., Hewer, T., Journault, N., & Kutsogiannis, D. (2018). Feasibility of a *Lactobacillus casei* drink in the intensive care unit for prevention of antibiotic associated diarrhea and *Clostridium difficile*. *Nutrients*, *10*(5), . https://doi.org/10.3390/nu10050539.

Arnbjerg, C. J., Vestad, B., Hov, J. R., Pedersen, K. K., Jespersen, S., Johannesen, H. H., et al. (2018). Effect of *Lactobacillus rhamnosus* GG supplementation on intestinal inflammation assessed by PET/MRI scans and gut microbiota composition in HIV-infected individuals. *Journal of Acquired Immune Deficiency Syndromes*, *78*(4), 450–457. https://doi.org/10.1097/QAI.0000000000001693.

Bagga, D., Reichert, J. L., Koschutnig, K., Aigner, C. S., Holzer, P., Koskinen, K., et al. (2018). Probiotics drive gut microbiome triggering emotional brain signatures. *Gut Microbes*, *9*(6), 1–11. https://doi.org/10.1080/19490976.2018.1460015.

Bakhshimoghaddam, F., Shateri, K., Sina, M., Hashemian, M., & Alizadeh, M. (2018). Daily consumption of synbiotic yogurt decreases liver steatosis in patients with nonalcoholic fatty liver disease: A randomized controlled clinical trial. *The Journal of Nutrition*, *148*(8), 1276–1284. https://doi.org/10.1093/jn/nxy088.

Barzegari, A., Kheyrolahzadeh, K., Mahdi, S., Khatibi, H., Sharifi, S., Memar, M. Y., et al. (2020). The battle of probiotics and their derivatives against biofilms. *Infection and drug resistance*. Dove Medical Press Ltd. https://doi.org/10.2147/IDR.S232982.

Bazanella, M., Maier, T. V., Clavel, T., Lagkouvardos, I., Lucio, M., Maldonado-Gòmez, M. X., et al. (2017). Randomized controlled trial on the impact of early-life intervention with bifidobacteria on the healthy infant fecal microbiota and metabolome. *American Journal of Clinical Nutrition*, *106*(5), 1274–1286. https://doi.org/10.3945/ajcn.117.157529.

Beltrán-Barrientos, L. M., González-Córdova, A. F., Hernández-Mendoza, A., Torres-Inguanzo, E. H., Astiazarán-García, H., Esparza-Romero, J., et al. (2018). Randomized double-blind controlled clinical trial of the blood pressure–lowering effect of fermented milk with *Lactococcus lactis*: A pilot study2. *Journal of Dairy Science*, *101*(4), 2819–2825. https://doi.org/10.3168/jds.2017-13189.

Bernhard, A. E., & Field, K. G. (2000). Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes. *Applied and Environmental Microbiology*, *66*(4), 1587–1594.

Biavati, B., & Mattarelli, P. (2006). The family *Bifidobacteriaceae*. In *The prokaryotes* (pp. 322–382). New York: Springer. https://doi.org/10.1007/0-387-30743-5_17.

Bottacini, F., Van Sinderen, D., & Ventura, M. (2017). Omics of bifidobacteria: Research and insights into their health-promoting activities. *Biochemical Journal*, *474*(24), 4137–4152. https://doi.org/10.1042/BCJ20160756.

Brodmann, T., Endo, A., Gueimonde, M., Vinderola, G., Kneifel, W., de Vos, W. M., et al. (2017). Safety of novel microbes for human consumption: Practical examples of assessment in the European Union. *Frontiers in Microbiology*, *8*(Sep), 1725. https://doi.org/10.3389/fmicb.2017.01725.

Candy, D. C. A., Van Ampting, M. T. J., Oude Nijhuis, M. M., Wopereis, H., Butt, A. M., Peroni, D. G., et al. (2018). A synbiotic-containing amino-acid-based formula improves gut microbiota in non-IgE-mediated allergic infants. *Pediatric Research*, *83*(3), 677–686. https://doi.org/10.1038/pr.2017.270.

Cani, P. D. (2018). Human gut microbiome: Hopes, threats and promises. *Gut*, *67*(9), 1716–1725. https://doi.org/10.1136/gutjnl-2018-316723.

Capece, A., Romaniello, R., Pietrafesa, A., Siesto, G., Pietrafesa, R., Zambuto, M., et al. (2018). Use of *Saccharomyces cerevisiae* var. *boulardii* in co-fermentations with *S. cerevisiae* for the production of craft beers with potential healthy value-added. *International Journal of Food Microbiology*, *284*, 22–30. https://doi.org/10.1016/j.ijfoodmicro.2018.06.028.

Cárdenas, N., Martín, V., Arroyo, R., López, M., Carrera, M., Badiola, C., et al. (2018). Prevention of recurrent acute otitis media in children through the use of *Lactobacillus salivarius* PS7, a target-specific probiotic strain. *Nutrients*, *11*(2), . https://doi.org/10.3390/nu11020376.

Carstensen, J. W., Chehri, M., Schønning, K., Rasmussen, S. C., Anhøj, J., Godtfredsen, N. S., et al. (2018). Use of prophylactic *Saccharomyces boulardii* to prevent *Clostridium difficile* infection in hospitalized patients: A controlled prospective intervention study. *European Journal of Clinical Microbiology and Infectious Diseases*, *37*(8), 1431–1439. https://doi.org/10.1007/s10096-018-3267-x.

Cencic, A. (2012). Can functional cell models Replace Laboratory Animals in Biomedical Research? *Journal of Bioanalysis & Biomedicine*, *04*(03), e105. https://doi.org/10.4172/1948-593X.1000e105.

Chang, C. J., Lin, T. L., Tsai, Y. L., Wu, T. R., Lai, W. F., Lu, C. C., et al. (2019). Next generation probiotics in disease amelioration. *Journal of Food and Drug Analysis*, *27*(3), 615–622. https://doi.org/10.1016/j.jfda.2018.12.011.

Chen, J., Cai, W., & Feng, Y. (2007). Development of intestinal bifidobacteria and lactobacilli in breast-fed neonates. *Clinical Nutrition*, *26*(5), 559–566. https://doi.org/10.1016/j.clnu.2007.03.003.

Chen, L., Xu, W., Lee, A., He, J., Huang, B., Zheng, W., et al. (2018). The impact of *Helicobacter pylori* infection, eradication therapy and probiotic supplementation on gut microenvironment homeostasis: An open-label, randomized clinical trial. *EBioMedicine*, *35*, 87–96. https://doi.org/10.1016/j.ebiom.2018.08.028.

Chong, H. X., Yusoff, N. A. A., Hor, Y. Y., Lew, L. C., Jaafar, M. H., Choi, S. B., et al. (2019). *Lactobacillus plantarum* DR7 alleviates stress and anxiety in adults: A randomised, double-blind, placebo-controlled study. *Beneficial Microbes*, *10*(4), 355–373. https://doi.org/10.3920/BM2018.0135.

Clarke, G., Stilling, R. M., Kennedy, P. J., Stanton, C., Cryan, J. F., & Dinan, T. G. (2014). Minireview: Gut microbiota: The neglected endocrine organ. *Molecular Endocrinology*, *28*(8), 1221–1238. https://doi.org/10.1210/me.2014-1108.

Crane, J., Barthow, C., Mitchell, E. A., Stanley, T. V., Purdie, G., Rowden, J., et al. (2018). Is yoghurt an acceptable alternative to raw milk for reducing eczema and allergy in infancy? *Clinical and Experimental Allergy*, *48*(5), 604–606. https://doi.org/10.1111/cea.13121.

Culpepper, T., Rowe, C. C., Rusch, C. T., Burns, A. M., Federico, A. P., Girard, S. A., et al. (2019). Three probiotic strains exert different effects on plasma bile acid profiles in healthy obese adults: Randomised, double-blind placebo-controlled crossover study. *Beneficial Microbes*, *10*(5), 497–509. https://doi.org/10.3920/BM2018.0151.

De Andrés, J., Manzano, S., García, C., Rodríguez, J. M., Espinosa-Martos, I., & Jiménez, E. (2018). Modulatory effect of three probiotic strains on infants' gut microbial composition and immunological parameters on a placebo-controlled, double-blind, randomised study. *Beneficial Microbes*, *9*(4), 573–584. https://doi.org/10.3920/BM2017.0132.

de Cássia Stampini Oliveira Lopes, R., Theodoro, J. M. V., da Silva, B. P., Queiroz, V. A. V., de Castro Moreira, M. E., Mantovani, H. C., et al. (2019). Synbiotic meal decreases uremic toxins in hemodialysis individuals: A placebo-controlled trial. *Food Research International*, *116*, 241–248. https://doi.org/10.1016/j.foodres.2018.08.024.

Dickerson, F., Adamos, M., Katsafanas, E., Khushalani, S., Origoni, A., Savage, C., et al. (2018). Adjunctive probiotic microorganisms to prevent rehospitalization in patients with acute mania: A randomized controlled trial. *Bipolar Disorders*, *20*(7), 614–621. https://doi.org/10.1111/bdi.12652.

Dore, M. P., Bibbò, S., Loria, M., Salis, R., Manca, A., Pes, G. M., et al. (2019). Twice-a-day PPI, tetracycline, metronidazole quadruple therapy with Pylera® or *Lactobacillus reuteri* for treatment naïve or for retreatment of *Helicobacter pylori*. Two randomized pilot studies. *Helicobacter*, *24*(6), e12659. https://doi.org/10.1111/hel.12659.

Dubernet, S., Desmasures, N., & Guéguen, M. (2002). A PCR-based method for identification of lactobacilli at the genus level. *FEMS Microbiology Letters*, *214*(2), 271–275.

Dunne, C., O'Mahony, L., Murphy, L., Thornton, G., Morrissey, D., O'Halloran, S., et al. (2001). In vitro selection criteria for probiotic bacteria of human origin: Correlation with in vivo findings. *American Journal of Clinical Nutrition*, *73*(2 Suppl.), 386S–392S. https://doi.org/10.1093/ajcn/73.2.386s.

Durack, J., Kimes, N. E., Lin, D. L., Rauch, M., McKean, M., McCauley, K., et al. (2018). Delayed gut microbiota development in high-risk for asthma infants is temporarily modifiable by *Lactobacillus* supplementation. *Nature Communications*, *9*(1), 1–9. https://doi.org/10.1038/s41467-018-03157-4.

Eggers, S., Barker, A. K., Valentine, S., Hess, T., Duster, M., & Safdar, N. (2018). Effect of *Lactobacillus rhamnosus* HN001 on carriage of *Staphylococcus aureus*: Results of the impact of probiotics for reducing infections in veterans (IMPROVE) study. *BMC Infectious Diseases*, *18*(1), 129. https://doi.org/10.1186/s12879-018-3028-6.

European Pharmacopoeia. (2019). *European Pharmacopoeia* (9th ed.). Council of Europe.

Faghihi, A. H., Agah, S., Masoudi, M., Ghafoori, S. M. S., & Eshraghi, A. (2015). Efficacy of probiotic *Escherichia coli* Nissle 1917 in patients with irritable bowel syndrome: A double blind placebo-controlled randomized trial. *Acta Medica Indonesiana*, *47*(3), 201–208.

FAO/WHO. (2001). *Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria*. .

FAO/WHO. (2002). Guidelines for the evaluation of probiotics in food. *Joint FAO/WHO Working Group report on Drafting Guidelines for the evaluation of probiotics in food*. London, Ontario, Canada: FAO/WHO. April 30 and May 1, 2002.

Farrokhian, A., Raygan, F., Soltani, A., Tajabadi-Ebrahimi, M., Sharifi Esfahani, M., Karami, A. A., et al. (2019). The effects of synbiotic supplementation on carotid intima-media thickness, biomarkers of inflammation, and oxidative stress in people with overweight, diabetes, and coronary heart disease: A randomized, double-blind, placebo-controlled trial. *Probiotics and Antimicrobial Proteins*, *11*(1), 133–142. https://doi.org/10.1007/s12602-017-9343-1.

Fijan, S. (2014). Microorganisms with claimed probiotic properties: An overview of recent literature. *International Journal of Environmental Research and Public Health*, *11*(5), 4745–4767. https://doi.org/10.3390/ijerph110504745.

Fijan, S., Frauwallner, A., Langerholc, T., Krebs, B., ter Haar (née Younes), J. A., Heschl, A., et al. (2019a). Efficacy of using probiotics with antagonistic activity against pathogens of wound infections: An integrative review of literature. *BioMed Research International*, *2019*(7585486), 1–21. https://doi.org/10.1155/2019/7585486.

Fijan, S., Frauwallner, A., Varga, L., Langerholc, T., Rogelj, I., Lorber, M., et al. (2019b). Health professionals' knowledge of probiotics: An international survey. *International Journal of Environmental Research and Public Health*, *16*(17), 3128. https://doi.org/10.3390/ijerph16173128.

Fijan, S., Šulc, D., & Steyer, A. (2018). Study of the in vitro antagonistic activity of various single-strain and multi-strain probiotics against *Escherichia coli*. *International Journal of Environmental Research and Public Health*, *15*(7), 1539. https://doi.org/10.3390/ijerph15071539.

Galofré, M., Palao, D., Vicario, M., Nart, J., & Violant, D. (2018). Clinical and microbiological evaluation of the effect of *Lactobacillus reuteri* in the treatment of mucositis and peri-implantitis: A triple-blind randomized clinical trial. *Journal of Periodontal Research*, *53*(3), 378–390. https://doi.org/10.1111/jre.12523.

Gerasimov, S., Gantzel, J., Dementieva, N., Schevchenko, O., Tsitsura, O., Guta, N., et al. (2018). Role of *Lactobacillus rhamnosus* (FloraActive™) 19070-2 and *Lactobacillus reuteri* (FloraActive™) 12246 in infant colic: A randomized dietary study. *Nutrients*, *10*(12), 1975. https://doi.org/10.3390/nu10121975.

Ghasemi, E., Mazaheri, R., & Tahmourespour, A. (2017). Effect of probiotic yogurt and xylitol-containing chewing gums on salivary *S. mutans* count. *Journal of Clinical Pediatric Dentistry*, *41*(4), 257–263. https://doi.org/10.17796/1053-4628-41.4.257.

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., et al. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology*, *14*(8), 491–502. https://doi.org/10.1038/nrgastro.2017.75.

Gillor, O., Etzion, A., & Riley, M. A. (2008). The dual role of bacteriocins as anti- and probiotics. *Applied Microbiology and Biotechnology*, *81*(4), 591–606. https://doi.org/10.1007/s00253-008-1726-5.

Gomi, A., Yamaji, K., Watanabe, O., Yoshioka, M., Miyazaki, K., Iwama, Y., et al. (2018). *Bifidobacterium bifidum* YIT 10347 fermented milk exerts beneficial effects on gastrointestinal discomfort and symptoms in healthy adults: A double-blind, randomized, placebo-controlled study. *Journal of Dairy Science*, *101*(6), 4830–4841. https://doi.org/10.3168/jds.2017-13803.

Haghshenas, B., Nami, Y., Almasi, A., Abdullah, N., Radiah, D., Rosli, R., et al. (2017). Isolation and characterization of probiotics from dairies. *Iranian Journal of Microbiology*, *9*(4), 234–243.

Hajifaraji, M., Jahanjou, F., Abbasalizadeh, F., Aghamohammadzadeh, N., Abbasi, M. M., & Dolatkhah, N. (2018). Effect of probiotic supplements in women with gestational diabetes mellitus on inflammation and oxidative stress biomarkers: A randomized clinical trial. *Asia Pacific Journal of Clinical Nutrition*, *27*(3), 581–591. https://doi.org/10.6133/apjcn.082017.03.

Håkansson, Å., Aronsson, C. A., Brundin, C., Oscarsson, E., Molin, G., & Agardh, D. (2019). Effects of *Lactobacillus plantarum* and *Lactobacillus paracasei* on the peripheral immune response in children with celiac disease autoimmunity: A randomized, double-blind, placebo-controlled clinical trial. *Nutrients*, *11*(8), 1925. https://doi.org/10.3390/nu11081925.

Hammes, W. P., & Vogel, R. F. (1995). The genus *Lactobacillus*. In *The genera of lactic acid bacteria* (pp. 19–54). US: Springer. https://doi.org/10.1007/978-1-4615-5817-0_3.

Hatanaka, M., Yamamoto, K., Suzuki, N., Iio, S., Takara, T., Morita, H., et al. (2018). Effect of *Bacillus subtilis* C-3102 on loose stools in healthy volunteers. *Beneficial Microbes*, *9*(3), 357–365. https://doi.org/10.3920/BM2017.0103.

He, C. X., Kong, F. T., Liang, F., Wang, K. X., Li, H., Liu, Y. L., et al. (2019). Influence of different timing of *Saccharomyces boulardii* combined with bismuth quadruple therapy for *Helicobacter pylori* eradication. *National Medical Journal of China*, *99*(22), 1731–1734. https://doi.org/10.3760/cma.j.issn.0376-2491.2019.22.010.

He, X., Zeng, Q., Puthiyakunnon, S., Zeng, Z., Yang, W., Qiu, J., et al. (2017). *Lactobacillus rhamnosus* GG supernatant enhance neonatal resistance to systemic *Escherichia coli* K1 infection by accelerating development of intestinal defense. *Scientific Reports*, *7*(1), 43305. https://doi.org/10.1038/srep43305.

Herman, L., Chemaly, M., Cocconcelli, P. S., Fernandez, P., Klein, G., Peixe, L., et al. (2019). The qualified presumption of safety assessment and its role in EFSA risk evaluations: 15 years past. *FEMS Microbiology Letters*, *366*(1), . https://doi.org/10.1093/FEMSLE/FNY260.

Hibberd, A. A., Yde, C. C., Ziegler, M. L., Honoré, A. H., Saarinen, A. T., Lahtinen, S., et al. (2019). Probiotic or synbiotic alters the gut microbiota and metabolism in a randomised controlled trial of weight management in overweight adults. *Beneficial Microbes*, *10*(2), 121–135.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., et al. (2014). The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*, *11*(8), 506–514. https://doi.org/10.1038/nrgastro.2014.66.

Hsieh, M. C., Tsai, W. H., Jheng, Y. P., Su, S. L., Wang, S. Y., Lin, C. C., et al. (2018). The beneficial effects of *Lactobacillus reuteri* ADR-1 or ADR-3 consumption on type 2 diabetes mellitus: A randomized, double-blinded, placebo-controlled trial. *Scientific Reports*, *8*(1), . https://doi.org/10.1038/s41598-018-35014-1.

Huang, C. F., Chie, W. C., & Wang, I. J. (2018a). Efficacy of *Lactobacillus* administration in school-age children with asthma: A randomized, placebo-controlled trial. *Nutrients*, *10*(11), 1678. https://doi.org/10.3390/nu10111678.

Huang, W. C., Hsu, Y. J., Li, H., Kan, N. W., Chen, Y. M., Lin, J. S., et al. (2018b). Effect of *Lactobacillus plantarum* TWK10 on improving endurance performance in humans. *Chinese Journal of Physiology*, *61*(3), 163–170. https://doi.org/10.4077/CJP.2018.BAH587.

Hwang, Y. H., Park, S., Paik, J. W., Chae, S. W., Kim, D. H., Jeong, D. G., et al. (2019). Efficacy and safety of *Lactobacillus plantarum* C29-fermented soybean (DW2009) in individuals with mild cognitive impairment: A 12-week, multi-center, randomized, double-blind, placebo-controlled clinical trial. *Nutrients*, *11*(2), . https://doi.org/10.3390/nu11020305.

Inoue, T., Kobayashi, Y., Mori, N., Sakagawa, M., Xiao, J. Z., Moritani, T., et al. (2018). Effect of combined bifidobacteria supplementation and resistance training on cognitive function, body composition and bowel habits of healthy elderly subjects. *Beneficial Microbes*, *9*(6), 843–853. https://doi.org/10.3920/BM2017.0193.

Invernici, M. M., Salvador, S. L., Silva, P. H. F., Soares, M. S. M., Casarin, R., Palioto, D. B., et al. (2018). Effects of *Bifidobacterium* probiotic on the treatment of chronic periodontitis: A randomized clinical trial. *Journal of Clinical Periodontology*, *45*(10), 1198–1210. https://doi.org/10.1111/jcpe.12995.

Jena, R., Choudhury, P. K., Puniya, A. K., & Tomar, S. K. (2017). Isolation and species delineation of genus *Bifidobacterium* using PCR-RFLP of partial hsp60 gene fragment. *LWT – Food Science and Technology*, *80*, 286–293. https://doi.org/10.1016/j.lwt.2017.02.032.

Karamali, M., Nasiri, N., Taghavi Shavazi, N., Jamilian, M., Bahmani, F., Tajabadi-Ebrahimi, M., et al. (2018). The effects of synbiotic supplementation on pregnancy outcomes in gestational diabetes. *Probiotics and Antimicrobial Proteins*, *10*(3), 496–503. https://doi.org/10.1007/s12602-017-9313-7.

Khalkhali, S., & Mojgani, N. (2017). Bacteriocinogenic potential and virulence traits of *Enterococcus faecium* and *E. faecalis* isolated from human milk. *Iranian Journal of Microbiology*, *9*(4), 224–233.

Kim, J., Yun, J. M., Kim, M. K., Kwon, O., & Cho, B. (2018). *Lactobacillus gasseri* BNR17 supplementation reduces the visceral fat accumulation and waist circumference in obese adults: A randomized, double-blind, placebo-controlled trial. *Journal of Medicinal Food*, *21*(5), 454–461. https://doi.org/10.1089/jmf.2017.3937.

Kinoshita, T., Maruyama, K., Suyama, K., Nishijima, M., Akamatsu, K., Jogamoto, A., et al. (2019). The effects of OLL1073R-1 yogurt intake on influenza incidence and immunological markers among women healthcare workers: A randomized controlled trial. *Food & Function*, *10*(12), 8129–8136. https://doi.org/10.1039/c9fo02128k.

Kobayashi, Y., Kuhara, T., Oki, M., & Xiao, J. Z. (2019). Effects of *Bifidobacterium breve* a1 on the cognitive function of older adults with memory complaints: A randomised, double-blind, placebo-controlled trial. *Beneficial Microbes*, *10*(5), 511–520. https://doi.org/10.3920/BM2018.0170.

Komano, Y., Shimada, K., Naito, H., Fukao, K., Ishihara, Y., Fujii, T., et al. (2018). Efficacy of heat-killed *Lactococcus lactis* JCM 5805 on immunity and fatigue during consecutive high intensity exercise in male athletes: A randomized, placebo-controlled, double-blinded trial. *Journal of the International Society of Sports Nutrition*, *15*(1), 39. https://doi.org/10.1186/s12970-018-0244-9.

Korcz, E., Kerényi, Z., & Varga, L. (2018). Dietary fibers, prebiotics, and exopolysaccharides produced by lactic acid bacteria: Potential health benefits with special regard to cholesterol-lowering effects. *Food and Function*, *9*(6), 3057–3068. https://doi.org/10.1039/c8fo00118a.

Korpela, K., Salonen, A., Vepsäläinen, O., Suomalainen, M., Kolmeder, C., Varjosalo, M., et al. (2018). Probiotic supplementation restores normal microbiota composition and function in antibiotic-treated and in caesarean-born infants. *Microbiome*, *6*(1), 182. https://doi.org/10.1186/s40168-018-0567-4.

Krasowska, A., Murzyn, A., Dyjankiewicz, A., Łukaszewicz, M., & Dziadkowiec, D. (2009). The antagonistic effect of *Saccharomyces boulardii* on *Candida albicans* filamentation, adhesion and biofilm formation. *FEMS Yeast Research*, *9*(8), 1312–1321. https://doi.org/10.1111/j.1567-1364.2009.00559.x.

Kusumo, P. D., Bela, B., Wibowo, H., Munasir, Z., & Surono, I. S. (2019). *Lactobacillus plantarum* IS-10506 supplementation increases faecal sIgA and immune response in children younger than two years. *Beneficial Microbes*, *10*(3), 245–252. https://doi.org/10.3920/BM2017.0178.

Lai, H. H., Chiu, C. H., Kong, M. S., Chang, C. J., & Chen, C. C. (2019). Probiotic *Lactobacillus casei*: Effective for managing childhood diarrhea by altering gut microbiota and attenuating fecal inflammatory markers. *Nutrients*, *11*(5), . https://doi.org/10.3390/nu11051150.

Lau, A. S. Y., Yanagisawa, N., Hor, Y. Y., Lew, L. C., Ong, J. S., Chuah, L. O., et al. (2018). *Bifidobacterium longum* BB536 alleviated upper respiratory illnesses and modulated gut microbiota profiles in Malaysian pre-school children. *Beneficial Microbes*, *9*(1), 61–70. https://doi.org/10.3920/BM2017.0063.

Lau, C. S., & Chamberlain, R. S. (2016). Probiotics are effective at preventing *Clostridium difficile*-associated diarrhea: A systematic review and meta-analysis. *International Journal of General Medicine*, *9*, 27. https://doi.org/10.2147/IJGM.S98280.

Laue, C., Papazova, E., Liesegang, A., Pannenbeckers, A., Arendarski, P., Linnerth, B., et al. (2018). Effect of a yoghurt drink containing *Lactobacillus* strains on bacterial vaginosis in women – A double-blind, randomised, controlled clinical pilot trial. *Beneficial Microbes*, *9*(1), 35–50. https://doi.org/10.3920/BM2017.0018.

Lee, X., Vergara, C., & Lozano, C. P. (2019). Severity of Candida-associated denture stomatitis is improved in institutionalized elders who consume *Lactobacillus rhamnosus* SP1. *Australian Dental Journal*, *64*(3), 229–236. https://doi.org/10.1111/adj.12692.

Lei, M., Guo, C., Wang, Y., Hua, L., Xue, S., Yu, D., et al. (2018). Oral administration of probiotic *Lactobacillus casei* Shirota relieves pain after single rib fracture: A randomized double-blind, placebo-controlled clinical trial. *Asia Pacific Journal of Clinical Nutrition*, *27*(6), 1252–1257. https://doi.org/10.6133/apjcn.201811_27(6).0012.

Linn, Y. H., Thu, K. K., & Win, N. H. H. (2019). Effect of probiotics for the prevention of acute radiation-induced diarrhoea among cervical cancer patients: A randomized double-blind placebo-controlled study. *Probiotics and Antimicrobial Proteins*, *11*(2), 638–647. https://doi.org/10.1007/s12602-018-9408-9.

Liu, Y., Alookaran, J., & Rhoads, J. (2018). Probiotics in autoimmune and inflammatory disorders. *Nutrients*, *10*(10), 1537. https://doi.org/10.3390/nu10101537.

Liu, Y. W., Liong, M. T., Chung, Y. C. E., Huang, H. Y., Peng, W. S., Cheng, Y. F., et al. (2019). Effects of *Lactobacillus plantarum* PS128 on children with autism spectrum disorder in Taiwan: A randomized, double-blind, placebo-controlled trial. *Nutrients*, *11*(4), 820. https://doi.org/10.3390/nu11040820.

Maldonado Galdeano, C., Cazorla, S. I., Lemme Dumit, J. M., Vélez, E., & Perdigón, G. (2019). Beneficial effects of probiotic consumption on the immune system. *Annals of Nutrition and Metabolism*, *74*(2), 115–124. https://doi.org/10.1159/000496426.

Malik, M., Suboc, T. M., Tyagi, S., Salzman, N., Wang, J., Ying, R., et al. (2018). *Lactobacillus plantarum* 299v supplementation improves vascular endothelial function and reduces inflammatory biomarkers in men with stable coronary artery disease. *Circulation Research*, *123*(9), 1091–1102. https://doi.org/10.1161/CIRCRESAHA.118.313565.

Mantaring, J., Benyacoub, J., Destura, R., Pecquet, S., Vidal, K., Volger, S., et al. (2018). Effect of maternal supplement beverage with and without probiotics during pregnancy and lactation on maternal and infant health: A randomized controlled trial in the Philippines. *BMC Pregnancy and Childbirth*, *18*(1), 193. https://doi.org/10.1186/s12884-018-1828-8.

Manzhalii, E., Hornuss, D., & Stremmel, W. (2016). Intestinal-borne dermatoses significantly improved by oral application of *Escherichia coli* Nissle 1917. *World Journal of Gastroenterology*, *22*(23), 5415–5421. https://doi.org/10.3748/wjg.v22.i23.5415.

Maragkoudaki, M., Chouliaras, G., Moutafi, A., Thomas, A., Orfanakou, A., & Papadopoulou, A. (2018). Efficacy of an oral rehydration solution enriched with *Lactobacillus reuteri* DSM 17938 and Zinc in the management of acute diarrhoea in infants: A randomized, double-blind, placebo-controlled trial. *Nutrients*, *10*(9), 1189. https://doi.org/10.3390/nu10091189.

Martín, V., Cárdenas, N., Ocaña, S., Marín, M., Arroyo, R., Beltrán, D., et al. (2019). Rectal and vaginal eradication of *Streptococcus agalactiae* (GBS) in pregnant women by using *Lactobacillus salivarius* CECT 9145, a target-specific probiotic strain. *Nutrients*, *11*(4), 810. https://doi.org/10.3390/nu11040810.

Massip, C., Branchu, P., Bossuet-Greif, N., Chagneau, C. V., Gaillard, D., Martin, P., et al. (2019). Deciphering the interplay between the genotoxic and probiotic activities of *Escherichia coli* Nissle 1917. *PLoS Pathogens*, *15*(9), e1008029. https://doi.org/10.1371/journal.ppat.1008029.

Matsumoto, M., Kitada, Y., & Naito, Y. (2019). Endothelial function is improved by inducing microbial polyamine production in the Gut: A randomized placebo-controlled trial. *Nutrients*, *11*(5), 1188. https://doi.org/10.3390/nu11051188.

Mattarelli, P., Felis, G. E., Pot, B., Holzapfel, W. H., & Franz, C. M. A. P. (2020). International Committee on Systematics of Prokaryotes Subcommittee on the taxonomy of *Bifidobacterium*, *Lactobacillus* and related organisms Minutes of the closed meeting, 20 June 2019, Prague, Czech Republic. *International Journal of Systematic and Evolutionary Microbiology*, *70*, 2949–2951. https://doi.org/10.1099/ijsem.0.004104.

Mattarelli, P., Holzapfel, W., Franz, C. M. A. P., Endo, A., Felis, G. E., Hammes, W., et al. (2014). Recommended minimal standards for description of new taxa of the genera *Bifidobacterium*, *Lactobacillus* and related genera. *International Journal of Systematic and Evolutionary Microbiology*, *64*(4), 1434–1451. https://doi.org/10.1099/ijs.0.060046-0.

McFarland, L. V. (2020). Efficacy of single-strain probiotics versus multi-strain mixtures: Systematic review of strain and disease specificity. *Digestive Diseases and Sciences*, 1–11. https://doi.org/10.1007/s10620-020-06244-z.

McMillan, A., Rulisa, S., Gloor, G. B., Macklaim, J. M., Sumarah, M., & Reid, G. (2018). Pilot assessment of probiotics for pregnant women in Rwanda. *PLoS One*, *13*(6), e0195081. https://doi.org/10.1371/journal.pone.0195081.

Milani, C., Duranti, S., Bottacini, F., Casey, E., Turroni, F., Mahony, J., et al. (2017). The first microbial colonizers of the human gut: Composition, activities, and health implications of the infant gut microbiota. *Microbiology and Molecular Biology Reviews*, *81*(4), . https://doi.org/10.1128/mmbr.00036-17.

Miyaoka, T., Kanayama, M., Wake, R., Hashioka, S., Hayashida, M., Nagahama, M., et al. (2018). *Clostridium butyricum* MIYAIRI 588 as adjunctive therapy for treatment-resistant major depressive disorder. *Clinical Neuropharmacology*, *41*(5), 151–155. https://doi.org/10.1097/WNF.0000000000000299.

Molnár, N., Gyenis, B., & Varga, L. (2005). Influence of a powdered Spirulina platensis biomass on acid production of lactococci in milk. *Milchwissenschaft*, *60*(4), 380–382.

Mortensen, B., Murphy, C., O'Grady, J., Lucey, M., Elsafi, G., Barry, L., et al. (2019). *Bifidobacterium breve* Bif195 protects against small-intestinal damage caused by acetylsalicylic acid in healthy volunteers. *Gastroenterology*, *157*(3), 637–646.e4. https://doi.org/10.1053/j.gastro.2019.05.008.

Nagpal, R., Wang, S., Ahmadi, S., Hayes, J., Gagliano, J., Subashchandrabose, S., et al. (2018). Human-origin probiotic cocktail increases short-chain fatty acid production via modulation of mice and human gut microbiome. *Scientific Reports*, *8*(1), 12649. https://doi.org/10.1038/s41598-018-30114-4.

Navarro-Lopez, V., Ramirez-Bosca, A., Ramon-Vidal, D., Ruzafa-Costas, B., Genoves-Martinez, S., Chenoll-Cuadros, E., et al. (2018). Effect of oral administration of a mixture of probiotic strains on SCORAD index and use of topical steroids in young patients with moderate atopic dermatitis a randomized clinical trial. *JAMA Dermatology*, *154*(1), 37–43. https://doi.org/10.1001/jamadermatol.2017.3647.

Nilsson, A. G., Sundh, D., Bäckhed, F., & Lorentzon, M. (2018). *Lactobacillus reuteri* reduces bone loss in older women with low bone mineral density: A randomized, placebo-controlled, double-blind, clinical trial. *Journal of Internal Medicine*, *284*(3), 307–317. https://doi.org/10.1111/joim.12805.

Nishida, K., Sawada, D., Kuwano, Y., Tanaka, H., & Rokutan, K. (2019). Health benefits of *Lactobacillus gasseri* CP2305 tablets in young adults exposed to chronic stress: A randomized, double-blind, placebo-controlled study. *Nutrients*, *11*(8), 1859. https://doi.org/10.3390/nu11081859.

Nishiyama, K., Kobayashi, T., Sato, Y., Watanabe, Y., Kikuchi, R., Kanno, R., et al. (2018). A double-blind controlled study to evaluate the effects of yogurt enriched with *Lactococcus lactis* 11/19-b1 and *Bifidobacterium lactis* on serum low-density lipoprotein level and antigen-specific interferon-γ releasing ability. *Nutrients*, *10*(11), 1778. https://doi.org/10.3390/nu10111778.

Oda, Y., Furutani, C., Mizota, Y., Wakita, A., Mimura, S., Kihara, T., et al. (2019). Effect of bovine milk fermented with *Lactobacillus rhamnosus* l8020 on periodontal disease in individuals with intellectual disability: A randomized clinical trial. *Journal of Applied Oral Science*, *27*, e20180564. https://doi.org/10.1590/1678-7757-2018-0564.

Okesene-Gafa, K. A. M., Li, M., McKinlay, C. J. D., Taylor, R. S., Rush, E. C., Wall, C. R., et al. (2019). Effect of antenatal dietary interventions in maternal obesity on pregnancy weight-gain and birthweight: Healthy Mums and Babies (HUMBA) randomized trial. *American Journal of Obstetrics and Gynecology*, *221*(2), 152.e1–152.e13. https://doi.org/10.1016/j.ajog.2019.03.003.

Ou, Y. C., Fu, H. C., Tseng, C. W., Wu, C. H., Tsai, C. C., & Lin, H. (2019). The influence of probiotics on genital high-risk human papilloma virus clearance and quality of cervical smear: A randomized placebo-controlled trial. *BMC Women's Health*, *19*(1), 103. https://doi.org/10.1186/s12905-019-0798-y.

Ozaki, K., Maruo, T., Kosaka, H., Mori, M., Mori, H., Yamori, Y., et al. (2018). The effects of fermented milk containing *Lactococcus lactis* subsp. *cremoris* FC on defaecation in healthy young Japanese women: A double-blind, placebo-controlled study. *International Journal of Food Sciences and Nutrition*, *69*(6), 762–769. https://doi.org/10.1080/09637486.2017.1417977.

Pahumunto, N., Piwat, S., Chankanka, O., Akkarachaneeyakorn, N., Rangsitsathian, K., & Teanpaisan, R. (2018). Reducing mutans streptococci and caries development by *Lactobacillus paracasei* SD1 in preschool children: A randomized placebo-controlled trial. *Acta Odontologica Scandinavica*, *76*(5), 331–337. https://doi.org/10.1080/00016357.2018.1453083.

Palma, E., Recine, N., Domenici, L., Giorgini, M., Pierangeli, A., & Panici, P. B. (2018). Long-term *Lactobacillus rhamnosus* BMX 54 application to restore a balanced vaginal ecosystem: A promising solution against HPV-infection. *BMC Infectious Diseases*, *18*(1), 13. https://doi.org/10.1186/s12879-017-2938-z.

Pandey, K. R., Naik, S. R., & Vakil, B. V. (2015). Probiotics, prebiotics and synbiotics – A review. *Journal of Food Science and Technology*, *52*(12), 7577–7587. https://doi.org/10.1007/s13197-015-1921-1.

Patil, R. U., Dastoor, P. P., & Unde, M. P. (2019). Comparative evaluation of antimicrobial effectiveness of probiotic milk and fluoride mouthrinse on salivary *Streptococcus mutans* counts and plaque scores in children—An in vivo experimental study. *Journal of Indian Society of Pedodontics and Preventive Dentistry*, *37*(4), 378–382. https://doi.org/10.4103/JISPPD.JISPPD_45_19.

Plummer, E. L., Bulach, D. M., Murray, G. L., Jacobs, S. E., Tabrizi, S. N., Garland, S. M., et al. (2018). Gut microbiota of preterm infants supplemented with probiotics: Sub-study of the ProPrems trial. *BMC Microbiology*, *18*(1), 184. https://doi.org/10.1186/s12866-018-1326-1.

Poonyam, P., Chotivitayatarakorn, P., & Vilaichone, R. K. (2019). High effective of 14-day high-dose PPI-bismuth-containing quadruple therapy with probiotics supplement for *Helicobacter pylori* eradication: A double blinded-randomized placebo-controlled study. *Asian Pacific Journal of Cancer Prevention*, *20*(9), 2859–2864. https://doi.org/10.31557/APJCP.2019.20.9.2859.

Pot, B., Felis, G. E., Bruyne, K., De Tsakalidou, E., Papadimitriou, K., Leisner, J., et al. (2014). The genus *Lactobacillus*. In *Lactic acid bacteria* (pp. 249–353). Chichester, UK: John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118655252.ch19.

Pot, B., Salvetti, E., Mattarelli, P., & Felis, G. E. (2019). The potential impact of the *Lactobacillus* name change: The results of an expert meeting organised by the Lactic Acid Bacteria Industrial Platform (LABIP). *Trends in Food Science and Technology*, *94*, 105–113. https://doi.org/10.1016/j.tifs.2019.07.006.

Preston, K., Krumian, R., Hattner, J., Demontigny, D., Stewart, M., & Gaddam, S. (2018). *Lactobacillus acidophilus* CL1285, *Lactobacillus casei* LBC80R and *Lactobacillus rhamnosus* CLR2 improve quality-of-life and IBS symptoms: A double-blind, randomised, placebo-controlled study. *Beneficial Microbes*, *9*(5), 697–706. https://doi.org/10.3920/BM2017.0105.

Pugh, J. N., Sparks, A. S., Doran, D. A., Fleming, S. C., Langan-Evans, C., Kirk, B., et al. (2019). Four weeks of probiotic supplementation reduces GI symptoms during a marathon race. *European Journal of Applied Physiology*, *119*(7), 1491–1501. https://doi.org/10.1007/s00421-019-04136-3.

Reid, G., Jass, J., Sebulsky, M. T., & McCormick, J. K. (2003). Potential uses of probiotics in clinical practice. *Clinical Microbiology Reviews*, *16*(4), 658–672. https://doi.org/10.1128/CMR.16.4.658-672.2003.

Reshes, G., Vanounou, S., Fishov, I., & Feingold, M. (2008). Cell shape dynamics in *Escherichia coli*. *Biophysical Journal*, *94*(1), 251–264. https://doi.org/10.1529/biophysj.107.104398.

Riezzo, G., Chimienti, G., Orlando, A., D'Attoma, B., Clemente, C., & Russo, F. (2019). Effects of long-term administration of *Lactobacillus reuteri* DSM-17938 on circulating levels of 5-HT and BDNF in adults with functional constipation. *Beneficial Microbes*, *10*(2), 137–147. https://doi.org/10.3920/BM2018.0050.

Roessner, C. A., Huang, K. X., Warren, M. J., Raux, E., & Scott, A. I. (2002). Isolation and characterization of 14 additional genes specifying the anaerobic biosynthesis of cobalamin (vitamin B12) in *Propionibacterium freudenreichii* (*P. shermanii*). *Microbiology*, *148*(6), 1845–1853. https://doi.org/10.1099/00221287-148-6-1845.

Rudzki, L., Ostrowska, L., Pawlak, D., Małus, A., Pawlak, K., Waszkiewicz, N., et al. (2019). Probiotic *Lactobacillus plantarum* 299v decreases kynurenine concentration and improves cognitive functions in patients with major depression: A double-blind, randomized, placebo controlled study. *Psychoneuroendocrinology*, *100*, 213–222. https://doi.org/10.1016/j.psyneuen.2018.10.010.

Russo, R., Superti, F., Karadja, E., & De Seta, F. (2019). Randomised clinical trial in women with Recurrent Vulvovaginal Candidiasis: Efficacy of probiotics and lactoferrin as maintenance treatment. *Mycoses*, *62*(4), 328–335. https://doi.org/10.1111/myc.12883.

Salas-Jara, M., Ilabaca, A., Vega, M., & García, A. (2016). Biofilm forming *Lactobacillus*: New challenges for the development of probiotics. *Microorganisms*, *4*(3), 35. https://doi.org/10.3390/microorganisms4030035.

Salem, I., Ramser, A., Isham, N., & Ghannoum, M. A. (2018). The gut microbiome as a major regulator of the gut-skin axis. *Frontiers in Microbiology*, *9*, 1459. https://doi.org/10.3389/fmicb.2018.01459.

Salvetti, E., Harris, H. M. B., Felis, G. E., & O'Toole, P. W. (2018). Comparative genomics of the genus *Lactobacillus* reveals robust phylogroups that provide the basis for reclassification. *Applied and Environmental Microbiology*, *84*(17), e00993-18. https://doi.org/10.1128/AEM.00993-18.

Savino, F., Galliano, I., Garro, M., Savino, A., Daprà, V., Montanari, P., et al. (2018). Regulatory T cells and Toll-like receptor 2 and 4 mRNA expression in infants with colic treated with *Lactobacillus reuteri* DSM17938. *Beneficial Microbes*, *9*(6), 917–925. https://doi.org/10.3920/BM2017.0194.

Schmidt, R. M., Pilmann Laursen, R., Bruun, S., Larnkjær, A., Mølgaard, C., Michaelsen, K. F., et al. (2019). Probiotics in late infancy reduce the incidence of eczema: A randomized controlled trial. *Pediatric Allergy and Immunology*, *30*(3), 335–340. https://doi.org/10.1111/pai.13018.

Schrezenmeir, J., & de Vrese, M. (2001). Probiotics, prebiotics, and synbiotics—Approaching a definition. *American Journal of Clinical Nutrition*, *73*(2), 361s–364s. https://doi.org/10.1093/ajcn/73.2.361s.

Seddik, H., Boutallaka, H., Elkoti, I., Nejjari, F., Berraida, R., Berrag, S., et al. (2019). *Saccharomyces boulardii* CNCM I-745 plus sequential therapy for *Helicobacter pylori* infections: A randomized, open-label trial. *European Journal of Clinical Pharmacology*, *75*(5), 639–645. https://doi.org/10.1007/s00228-019-02625-0.

Shi, C. Z., Chen, H. Q., Liang, Y., Xia, Y., Yang, Y. Z., Yang, J., et al. (2014). Combined probiotic bacteria promotes intestinal epithelial barrier function in interleukin-10-gene-deficient mice. *World Journal of Gastroenterology*, *20*(16), 4636–4647. https://doi.org/10.3748/wjg.v20.i16.4636.

Shimizu, K., Yamada, T., Ogura, H., Mohri, T., Kiguchi, T., Fujimi, S., et al. (2018). Synbiotics modulate gut microbiota and reduce enteritis and ventilator-associated pneumonia in patients with sepsis: A randomized controlled trial. *Critical Care*, *22*(1), 239. https://doi.org/10.1186/s13054-018-2167-x.

Soman, R. J., & Swamy, M. V. (2019). A prospective, randomized, double-blind, placebo-controlled, parallel-group study to evaluate the efficacy and safety of SNZ TriBac, a three-strain *Bacillus* probiotic blend for undiagnosed gastrointestinal discomfort. *International Journal of Colorectal Disease*, *34*(11), 1971–1978. https://doi.org/10.1007/s00384-019-03416-w.

Sul, S. -Y., Kim, H. -J., Kim, T. -W., & Kim, H. -Y. (2007). Rapid identification of *Lactobacillus* and *Bifidobacterium* in probiotic products using multiplex PCR. *Journal of Microbiology and Biotechnology*, *17*(3), 490–495.

Sun, Q. H., Wang, H. Y., Sun, S. D., Zhang, X., & Zhang, H. (2019). Beneficial effect of probiotics supplements in reflux esophagitis treated with esomeprazole: A randomized controlled trial. *World Journal of Gastroenterology*, *25*(17), 2110–2121. https://doi.org/10.3748/wjg.v25.i17.2110.

Sun, Z., Harris, H. M. B., McCann, A., Guo, C., Argimón, S., Zhang, W., et al. (2015). Expanding the biotechnology potential of lactobacilli through comparative genomics of 213 strains and associated genera. *Nature Communications*, *6*(1), 8322. https://doi.org/10.1038/ncomms9322.

Tenorio-Jiménez, C., Martínez-Ramírez, M. J., Del Castillo-Codes, I., Arraiza-Irigoyen, C., Tercero-Lozano, M., Camacho, J., et al. (2019). *Lactobacillus reuteri* v3401 reduces inflammatory biomarkers and modifies the gastrointestinal microbiome in adults with metabolic syndrome: The PROSIR study. *Nutrients*, *11*(8), 1761. https://doi.org/10.3390/nu11081761.

Thankappan, B., Ramesh, D., Ramkumar, S., Natarajaseenivasan, K., & Anbarasu, K. (2015). Characterization of *Bacillus* spp. from the gastrointestinal tract of *Labeo rohita*—Towards to identify novel probiotics against fish pathogens. *Applied Biochemistry and Biotechnology*, *175*(1), 340–353. https://doi.org/10.1007/s12010-014-1270-y.

Twetman, S., Keller, M. K., Lee, L., Yucel-Lindberg, T., & Lynge Pedersen, A. M. (2018). Effect of probiotic lozenges containing *Lactobacillus reuteri* on oral wound healing: A pilot study. *Beneficial Microbes*, *9*(5), 691–696. https://doi.org/10.3920/BM2018.0003.

Vaisberg, M., Paixão, V., Almeida, E. B., Santos, J. M. B., Foster, R., Rossi, M., et al. (2019). Daily intake of fermented milk containing *Lactobacillus casei* shirota (lcs) modulates systemic and upper airways immune/inflammatory responses in marathon runners. *Nutrients*, *11*(7), 1678. https://doi.org/10.3390/nu11071678.

Varga, L., & Andok, T. (2018). Viability of bifidobacteria in soft-frozen ice cream supplemented with a *Saccharomyces cerevisiae* cell wall product. *Acta Alimentaria*, *47*(3), 387–392. https://doi.org/10.1556/066.2018.47.3.15.

Varga, L., Szigeti, J., & Csengeri, E. (2003). Effect of oligofructose on the microflora of an ABT-type fermented milk during refrigerated storage. *Scinapse*, *58*(1/2), 55–58.

Varga, L., Szigeti, J., & Gyenis, B. (2006). Influence of chicory inulin on the survival of microbiota of a probiotic fermented milk during refrigerated storage. *Annals of Microbiology*, *56*(2), 139–141. https://doi.org/10.1007/BF03174995.

Vitellio, P., Celano, G., Bonfrate, L., Gobbetti, M., Portincasa, P., & De Angelis, M. (2019). Effects of *Bifidobacterium longum* and *Lactobacillus rhamnosus* on gut microbiota in patients with lactose intolerance and persisting functional gastrointestinal symptoms: A randomised, double-blind, cross-over study. *Nutrients*, *11*(4), 886. https://doi.org/10.3390/nu11040886.

Vladareanu, R., Mihu, D., Mitran, M., Mehedintu, C., Boiangiu, A., Manolache, M., et al. (2018). New evidence on oral L. plantarum P17630 product in women with history of recurrent vulvovaginal candidiasis (RVVC): A randomized double-blind placebo-controlled study. *European Review for Medical Pharmacological Sciences*, *22*(1), 262–267.

Walter, J., Tannock, G. W., Tilsala-Timisjarvi, A., Rodtong, S., Loach, D. M., Munro, K., et al. (2000). Detection and identification of gastrointestinal *Lactobacillus* species by using denaturing gradient gel electrophoresis and species-specific PCR primers. *Applied and Environmental Microbiology*, *66*(1), 297–303.

Wan, L. Y. M., Chen, Z. J., Shah, N. P., & El-Nezami, H. (2016). Modulation of intestinal epithelial defense responses by probiotic bacteria. *Critical Reviews in Food Science and Nutrition*, *56*(16), 2628–2641. https://doi.org/10.1080/10408398.2014.905450.

Wang, H., Braun, C., Murphy, E. F., & Enck, P. (2019). *Bifidobacterium longum* 1714™ strain modulates brain activity of healthy volunteers during social stress. *American Journal of Gastroenterology*, *114*(7), 1152–1162. https://doi.org/10.14309/ajg.0000000000000203.

Wickens, K., Barthow, C., Mitchell, E. A., Kang, J., van Zyl, N., Purdie, G., et al. (2018). Effects of *Lactobacillus rhamnosus* HN001 in early life on the cumulative prevalence of allergic disease to 11 years. *Pediatric Allergy and Immunology*, *29*(8), 808–814. https://doi.org/10.1111/pai.12982.

Wittouck, S., Wuyts, S., Meehan, C. J., van Noort, V., & Lebeer, S. (2019). A genome-based species taxonomy of the *Lactobacillus* genus complex. *MSystems*, *4*(5), e00264-19. https://doi.org/10.1128/msystems.00264-19.

Xia, X., Chen, J., Xia, J., Wang, B., Liu, H., Yang, L., et al. (2018). Role of probiotics in the treatment of minimal hepatic encephalopathy in patients with HBV-induced liver cirrhosis. *Journal of International Medical Research*, *46*(9), 3596–3604. https://doi.org/10.1177/0300060518776064.

Yamamoto, Y., Saruta, J., Takahashi, T., To, M., Shimizu, T., Hayashi, T., et al. (2019). Effect of ingesting yogurt fermented with *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1 on influenza virus-bound salivary IgA in elderly residents of nursing homes: A randomized controlled trial. *Acta Odontologica Scandinavica*, *77*(7), 517–524. https://doi.org/10.1080/00016357.2019.1609697.

Yamanaka, H., Taniguchi, A., Tsuboi, H., Kano, H., & Asami, Y. (2019). Hypouricaemic effects of yoghurt containing *Lactobacillus gasseri* PA-3 in patients with hyperuricaemia and/or gout: A randomised, double-blind, placebo-controlled study. *Modern Rheumatology*, *29*(1), 146–150. https://doi.org/10.1080/14397595.2018.1442183.

Yoon, J. Y., Cha, J. M., Hong, S. S., Kim, H. K., Kwak, M. S., Jeon, J. W., et al. (2019). Fermented milk containing *Lactobacillus paracasei* and *Glycyrrhiza glabra* has a beneficial effect in patients with *Helicobacter pylori* infection: A randomized, double-blind, placebo-controlled study. *Medicine*, *98*(35), e16601. https://doi.org/10.1097/MD.0000000000016601.

Yoon, J. Y., Cha, J. M., Oh, J. K., Tan, P. L., Kim, S. H., Kwak, M. S., et al. (2018). Probiotics ameliorate stool consistency in patients with chronic constipation: A randomized, double-blind, placebo-controlled study. *Digestive Diseases and Sciences*, *63*(10), 2754–2764. https://doi.org/10.1007/s10620-018-5139-8.

Younes, J. A., Lievens, E., Hummelen, R., van der Westen, R., Reid, G., & Petrova, M. I. (2018). Women and their microbes: The unexpected friendship. *Trends in Microbiology*, *26*, 16–32. https://doi.org/10.1016/j.tim.2017.07.008.

Zaharuddin, L., Mokhtar, N. M., Muhammad Nawawi, K. N., & Raja Ali, R. A. (2019). A randomized double-blind placebo-controlled trial of probiotics in post-surgical colorectal cancer. *BMC Gastroenterology*, *19*(1), 131. https://doi.org/10.1186/s12876-019-1047-4.

Zheng, J., Wittouck, S., Salvetti, E., Franz, C. M. A. P., Harris, H. M. B., Mattarelli, P., et al. (2020). A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *International Journal of Systematic and Evolutionary Microbiology*, *70*(4), 2782–2858. https://doi.org/10.1099/ijsem.0.004107.

Zhou, L., & Foster, J. A. (2015). Psychobiotics and the gut-brain axis: In the pursuit of happiness. *Neuropsychiatric Disease and Treatment*, *11*, 715–723. https://doi.org/10.2147/NDT.S61997.

Chapter 2

Selection Criteria for Identifying Putative Probiont

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1 Introduction

Lactic acid bacteria (LAB) have been traditionally used as starter cultures in the preparation of fermented foods, particularly in dairy-based products, health benefits of which have been known since ancient times. During the turn of the last century, with the suggestion of Elie Metchnikoff that the ingestion of live microorganisms has a positive effect on the intestinal microflora and thereby improves human health and longevity, the concept of probiotic was proposed (Scott et al., 2017). Today, the concept of probiotics is more than 100-year-old, and these viable and beneficial microorganisms are now commercially marketed either in pure form as capsules and sachets or incorporated in food formulations (Pradhan, Mallappa, & Grover, 2020). The term "probiotic" has been derived from the Greek words "pro" (for) and "bios" (life). The term "probiotic" was first described in 1953 by Kollath. Later, in 1989, Fuller emphasized that probiotics are "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" (Fuller, 1989). In 2001 an expert panel of the FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization) evaluated the health and nutritional benefits of probiotics in foods. The probiotics were defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2001). This widely accepted scientific definition has also been incorporated by regulatory organizations worldwide into their local legal framework for probiotics (Vinderola, Reinheimer, & Salminen, 2019). Finally, in 2013, in a meeting organized by the ISAPP (International Scientific Association for Probiotics and Prebiotics), which was attended by the members of the FAO/WHO working group, minor grammatical corrections were made to the original definition of probiotics. Thus the original definition of probiotics was reworded as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill et al., 2014).

Although most traditional probiotics belong to well-known microbial groups (such as lactobacilli and bifidobacteria) with a long history of safe use, the probiotic market constantly requires the implementation and diversification of the available products. Although these groups are already known to be "core beneficial," there is increasing interest to identify novel probiotic candidates that are both different from LAB groups, such as yeast and *Bacillus*, and more specific LAB strains (de Melo Pereira, de Oliveira Coelho, Júnior, Thomaz-Soccol, & Soccol, 2018; Vinderola et al., 2019). Since there was no international regulation to validate the efficacy and safety of probiotic microorganisms, FAO/WHO published the "Guidelines for Evaluation of Probiotics in Food" in 2002 to establish these standards (FAO/WHO, 2002). In these guidelines the suggested selection criteria for probiotic candidates were based on different aspects such as functionality, safety, and technological characteristics. Functional aspects include the resistance to unfavorable conditions imposed by the human body, ability to adhere to the epithelium, immunomodulatory, antagonistic, and antimutagenic properties. The safety characteristics of probiotic candidates, such as the strain's origin (healthy human GI tract), pathogenicity, and antibiotic resistance, are evaluated. The probiotic candidates selected on the basis of their functionality and safety characteristics must be able to be manufactured under industrial conditions. Therefore the bacteria also have to survive and retain their

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00002-2
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functionality during storage, without inducing changes in the flavor of foods wherein they have been incorporated (Saarela, Mogensen, Fondén, Mättö, & Mattila-Sandholm, 2000; de Melo Pereira et al., 2018). The efficacy of a new probiotic strain selected using in vitro assays, focusing on their ability to survive and colonize in the gastrointestinal environment, must be confirmed by in vivo assays.

The relationship of microorganisms with the health and well-being of humans has made it essential to develop new strategies that evaluate the biological properties of probiotics, such as anticarcinogenic, antidepression, antiobesity, antidiabetic, and cholesterol-lowering activities (de Melo Pereira et al., 2018). Recently, increased attention has been paid to a new concept, termed next-generation probiotics (NGP), due to their health-related benefits and therapeutic applications (Liu, Zhang, Dong, Yuan, & Guop, 2009). Notably, the researchers, who have pointed out the potential of the application of NGP as drugs (live biotherapeutic products), have emphasized the necessity of significantly more stringent measures than traditional probiotics. In this context, comprehensive studies on the effectiveness of disease control, safety, physiology, genomics, metabolomics characteristics, drug susceptibility patterns and transfer of drug resistance genes, and potential virulence factors (VFs) are mandatory (Liu et al., 2009; de Melo Pereira et al., 2018).

The process of the selection of novel probiotics with predictable safety and beneficial clinical outcomes needs to be elucidated in detail. In this chapter, we aimed to provide a comprehensive theoretical guide to select probiotic candidates, considering both functional and safety aspects.

2 Probiotic microorganisms

Probiotic cultures that are used in commercially marketed products such as capsules, sachets, or incorporated in food formulations predominantly belong to the LAB group, certain yeasts (*Saccharomyces boulardii*), and *Bacillus* species. The LAB group, which is historically defined by the organisms' ability to ferment hexose sugars to lactate, constitute the major group of the healthy human gastrointestinal tract (GIT) microflora (Ruiz-Moyano et al., 2012; Pradhan et al., 2020). Because of their long history of safe use and rarely occurring mild public health problems, most of the LAB, such as *Lactobacillus*, *Lactococcus*, and *Bifidobacterium*, are known as "GRAS" (generally recognized as safe) (Floch, 2013). When reports favoring the probiotic properties of *Lactobacillus* and *Bifidobacterium* were assessed through metaanalyses, the results indicated that these genera were always effective in a strain-dependent manner against different microbiota-associated diseases (Vinderola, Gueimonde, Gomez-Gallego, Delfederico, & Salminen, 2017).

The genus *Lactobacillus*, which belongs to the phylum Firmicutes, is a rod-shaped, Gram-positive bacterium and comprises over 183 recognized species. The *Lactobacillus* species, which are used in several industrial processes, such as the manufacture of preservatives, acidulants, food flavorings, drugs, and cosmetics (König & Fröhlich, 2009), were the earliest discovered probiotics (de Melo Pereira et al., 2018). Lactobacilli (along with bifidobacteria) are among the first bacteria to colonize the infant mammalian gut and are the key components of beneficial interactions with their hosts. Therefore it is not surprising that this bacterial taxon has been a prominent source of probiotic strains. EFSA (European Food Safety Authority) has granted the status of qualified presumption of safety (QPS) to 36 species of *Lactobacillus* based on their safety evaluation criteria (Ricci et al., 2017). These species are as follows: *Lactobacillus acidophilus*, *Lb. amylolyticus*, *Lb. amylovorus*, *Lb. animalis*, *Lb. alimentarius*, *Lb. aviaries*, *Lb. brevis*, *Lb. buchneri*, *Lb. casei*, *Lb. cellobiosus*, *Lb. collinoides*, *Lb. coryniformis*, *Lb. crispatus*, *Lb. curvatus*, *Lb. delbrueckii*, *Lb. diolivorans*, *Lb. farciminis*, *Lb. fermentum*, *Lb. gallinarum*, *Lb. gasseri*, *Lb. helveticus*, *Lb. hilgardii*, *Lb. johnsonii*, *Lb. kefiranofaciens*, *Lb. kefiri*, *Lb. mucosae*, *Lb. panis*, *Lb. paracasei*, *Lb. paraplantarum*, *Lb. pentosus*, *Lb. plantarum*, *Lb. pontis*, *Lb. reuteri*, *Lb. rhamnosus*, *Lb. sakei*, *Lb. salivarius* and *Lb. sanfranciscensis* (Pradhan et al., 2020). One of the most widely studied probiotic strain is *Lb. rhamnosus* GG (LGG) ATCC 53103 that was originally isolated from the feces of a healthy adult (Gorbach, Doron, & Magro, 2017). The strain LGG not only meets the necessary characteristics for an ideal probiotic but is also known to produce a compound (bacteriocin) with antimicrobial activity against anaerobic bacteria such as *Clostridium*, *Bacteroides*, *Bifidobacterium*, *Escherichia coli*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, and *Salmonella* (Gorbach et al., 2017). On the other hand, the use of another strain (*Lb. acidophilus*) as probiotics has been supported by data showing its postgastric survival and persistence in feces (up to 10 days postingestion) in humans. Furthermore, the use of multiple probiotic strains is also common. The proprietary probiotic preparation VSL#3 (VSL Pharmaceuticals Inc., Fort Lauderdale, United States) comprises four strains of lactobacilli (*Lb. acidophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. casei*, and *Lb. plantarum*), three strains of bifidobacteria (*B. breve*, *B. longum*, and *B. infantis*), and one strain of *Streptococcus salivarius* subsp. *thermophilus* (Neish, 2017).

The members of the genus *Bifidobacterium* have been classified into phylum Actinobacteria, one of the largest phyla in the domain Bacteria. With a few exceptions the species are characterized by a high content of guanine and cytosine (range 42%–67%). This Gram-positive, catalase-negative, Y-shaped, or branched bacterium (termed "Bifidus") was first isolated

from the GIT of breast-fed infants by the French pediatrician Henry Tissier. These anaerobic, heterofermentative, nonmotile, nonspore forming, and nongas producing bacteria can metabolize glucose, galactose, lactose, and fructose (Russell, Ross, Fitzgerald, & Stanton, 2011). Although they are important components of the commensal bacterial populations of the vagina and oral cavity, bifidobacteria are predominantly located in the colon because of their anaerobic metabolism and sensitivity to oxygen (Quigley, 2017a; de Melo Pereira et al., 2018). To date, at least 30 species have been identified within the genus *Bifidobacterium*, among which 10 (*B. adolescentis*, *B. angulatum*, *B. bifidum*, *B. breve*, *B. catenulatum*, *B. dentium*, *B. gallicum*, *B. longum*, and *B. pseudocatenulatum*) have been recovered from humans (feces, vaginal, or oral cavity), and 17 from animals, 2 from residual waters, and 1 from fermented milk (Lee & O'Sullivan, 2010; Russell et al., 2011; Quigley, 2017a). Due to their relative richness in the gut of breast-fed infants and the associated lower rates of diarrhea in these infants compared to the bottle-fed ones, the probiotic potential of bifidobacteria has been noticed at the first instance. This observation has been further supported by experiments proving that the prebiotic properties of oligosaccharides in human breast milk promote the growth of bifidobacteria. *B. animalis* subsp. *animalis*, *B. animalis* subsp. *lactis*, *B. breve*, *B. longum* subsp. *infantis*, and *B. longum* subsp. *longum*, the homeostatic and beneficial effects of which are proven by laboratory data and clinical evidence and have been selected for use as probiotics (Quigley, 2017a, 2017b).

Yeasts, which constitute a large and heterogeneous class of eukaryotic microorganisms, are widespread in natural environments ranging from the human intestinal tract to plants, airborne particles, and food products (de Melo Pereira et al., 2018; Sena & Mansella, 2020). The results of the mycobiome studies indicated that strains of *Saccharomyces* occur in up to 96.8% of the samples. The high prevalence of *Saccharomyces* strains in the human GI tract is not surprising, since *Saccharomyces cerevisiae* has been consumed by humans for thousands of years in bread, beer, and other fermented foods and beverages (Sena & Mansella, 2020). On the other hand, the high contents of proteins, vitamin B, trace minerals, and various immune-stimulating compounds (proteases, β-glucans, and mannan oligosaccharides), associated with these organisms, indicate that they also have the potential to be used as a probiotic (Gil-Rodríguez, Carrascosa, & Requena, 2015; de Melo Pereira et al., 2018; Sena & Mansella, 2020). Some characteristic properties of yeast, such as nonsusceptibility to antibiotics and good tolerance for industrial processing conditions (such as lyophilization and high temperatures), can also be considered as advantages over bacterial probiotic candidates (Abdel-Rahman, Tashiro, & Sonomoto, 2013; de Melo Pereira et al., 2018). One well-characterized probiotic yeast based on the QPS status, *S. boulardii*, was isolated about a 100 years ago by a French scientist named Henri Boulard, from the peels of lychee (*Litchi chinensis*) and mangosteen (*Garcinia mangostana*) (Sena & Mansella, 2020).

The Gram-positive, aerobic, and endospore-forming *Bacillus* species are ubiquitous and are found mainly in soil, water, and food products of plant origin. *Bacillus* species are known to be metabolically highly active and can produce several useful enzymes and numerous antibiotics. Strains of *Bacillus* (*Bacillus clausii* and *B. subtilis*) are also good probiotic candidates in the food and pharmaceutical industry. Owing to their ability to form endospores, strains of *Bacillus* remain stable in probiotic products for much longer than conventional probiotics (Sumi, Yang, Yeo, & Hahm, 2015Schultz, Burton, & Chanyi, 2017). This feature is not only responsible for the longer shelf lives of probiotics but also spares most probiotics from the rigors of food processing, including those designed to deplete microorganisms, such as pasteurization (Melo et al., 2017; Schultz, Burton, & Chanyi, 2017). Although some species of *Bacillus*, including *B. cereus*, *B. anthracis*, *B. thuringiensis*, *B. pseudomycoides*, and *B. weihenstephanensis*, have a history of use in fermented foods mainly in Africa and Asia, they are known to produce enterotoxins, which represents a safety concern (Abdel-Rahman, Tashiro, & Sonomoto, 2013; Melo et al., 2017; Schultz, Burton, & Chanyi, 2017).

3 Requirements for the selection of probiotic strains

The selection of probiotic microorganisms requires a systematic approach. Based on several papers till date, we can infer that the theoretical basis of the selection of successful probiotics is based on three main aspects: safety, functionality, and technology. Basically, these traditional assessments with wide scientific consensus for being validated by preliminary in vitro assays are related to the ecological origin of bacteria, their tolerance to the hostile conditions in the stomach and the small intestine, and their ability to adhere to the intestinal surfaces (FAO/WHO, 2001, 2002). When these approaches are handled in more detail, the criteria such as the origin, taxonomic identification, GRAS status, nontoxic, and nonpathogenic characteristics of candidates are related to their safety. In this respect, characteristics such as stress tolerance (resistance to gastric juice, enzymes, and bile salts), adhesion ability (adherence and colonization potential), and antipathogenic activity (ability to compete with healthy microflora and production of antimicrobial metabolites) of probiotic candidates are related to their competitiveness. These requirements mandated by the FAO and WHO should be supported by in vivo trials and also supplemented with the following additional requirements: (1) industrial requirements [viability at high population densities (preferably 10^6–10^8 CFU/mL), food processing stress, storage-related stress, and provision of desirable organoleptic

qualities], (2) host-associated functional properties (anticancer, anticholesterol, antiobesity, antidiabetic, antidepressant, antianxiety, immunostimulant, and secretion of functional molecules), and (3) characterization of OMICS (genomics, transcriptomics, proteomics, and metabolomics) (Klaenhammer & Kullen, 1999; de Melo Pereira et al., 2018).

3.1 Survival during gastrointestinal transit

Survival during gastrointestinal transit is the primary selection criterion that is commonly used in research on probiotics. The candidate strains should cope with the stress conditions prevalent in the human body. Exposure of the probiotic candidates to stress conditions starts in the oral cavity, continues with the harsh environment of the stomach, and ends with the exposure to high concentrations of bile salts in the first portion of the small intestine. These stress conditions of the human body can be simulated in vitro by cultivating the potential probiotic strains at different pH in the presence of enzymes such as pepsin, lysozyme, and amylase; phenol; NaCl; Oxgall; porcine gastric juice; pancreatic acid; and taurodeoxycholic acid. Resistance to these compounds can be determined by the colony count or measurement of the absorbance at different time intervals (Divya, Varsha, & Nampoothiri, 2012; de Melo Pereira et al., 2018).

When the stress conditions of the human body are handled step-by-step, in the oral cavity, the candidate microorganism should be resistant to enzymes such as amylase and lysozyme. Generally, Gram-positive bacteria are known to be sensitive to lysozyme. However, some LAB groups that constitute most probiotics are more resistant than other Gram-positive bacteria (Fuller, 1989; de Melo Pereira et al., 2018). Before probiotics reach the small intestine and colonize the host in sufficient numbers (at least 10^6–10^7 CFU/g), they must survive in the acidic gastric environment. Resistance to gastric acidity has been determined in vitro using pure cultures of the potential probiotic strain exposed to hydrochloric acid solutions of low pH and containing pepsin and sodium chloride (Vinderola et al., 2017). Some probiotics, for example, *Lactobacillus* species, are known to be intrinsically resistant to acid. For example, LGG, which is a human-derived, commercial probiotic strain with recognized health benefits, is resistant to pH values as low as 2.5 for 4 hours (Jacobsen et al., 1999). Although there are differences between species and strains, it has been highlighted that organisms generally exhibit increased sensitivity at pH below 3.0 (Corcoran, Stanton, Fitzgerald, & Ross, 2005; Ronka et al., 2003). When the internal pH reaches a threshold value, bacterial cells die due to the inhibition of their cellular functions. A mechanism, known as F0F1-ATPase, protects the Gram-positive organisms against acidic conditions (Cotter & Hill, 2003). The function of F0F1-ATPase, a multiple-subunit enzyme consisting of a catalytic portion (F1) and an integral membrane portion (F0), is to act as a membranous channel for the translocation of protons, which leads to an increase in the intracellular pH at low extracellular pH (Sebald, Friedl, Schairer, & Hoppe, 1982). The intrinsic acid tolerance shown by lactobacilli can be explained by the presence of a constant gradient between extracellular and cytoplasmic pH, which is induced at low pH and regulated at the transcriptional level (Corcoran et al., 2005). However, several in vitro gastric transit studies have the following limitations.

Although in vitro assays expose probiotic candidates to a consistently low pH (ranging from 1.5 to 3), during the actual digestive process, the pH of the stomach is known to display a gradual increase in acidity (termed pH diminution) (Blanquet et al., 2004). At the same time, researchers have indicated that gastric emptying is a dynamic process and depends on the individual's age and the consistency of the food consumed (Martinez et al., 2011). Under in vivo conditions, not all probiotic cells are exposed to the same pH at the same time, and this stress gradient is expected to continue until cells enter the small intestine. Considering that the static in vitro experiments might be more inhibitory than real gastric digestion, the actual gastric environment should be simulated by a gradual and manual reduction in pH, to determine the survival ability of probiotic candidates under real conditions (Vinderola et al., 2017).

Studies have reported that the same probiotics behave differently in different food matrices, and the buffering capability of the food matrix enhances the probiotic survival within the GIT (Sumeri, Arike, Adamberg, & Paalme, 2008). These differences are related to variations in bile secretion between individuals and could also be explained by the contradictory results obtained between in vitro and in vivo experiments (Mathieu, Nguyen, Amine, & Lacroix, 2013). Although data indicating the enhancement of survival of lactobacilli by food components are limited, increased survival rates in acidic conditions in the presence of glucose have been reported. It has been suggested in in vitro experiments that the use of acidified MRS medium, as a rich medium, may protect bacteria by providing energy and metabolic precursors (Corcoran et al., 2005). In this context, it can be suggested that the physiology of bacteria grown on laboratory media may be different from those exposed to the gastrointestinal environment (Vinderola et al., 2017).

3.2 Adhesion to gut cells

The adhesion to and colonization of the intestinal cell surface have been considered to be essential prerequisites for the action of probiotics, after survival during gastrointestinal transit. Although adhesion of microbes to epithelial cells is a

rather complicated process, the adherence of probiotics has the following benefits: (1) adherent strains of probiotic bacteria are likely to persist longer in the intestinal tract, (2) they have a better chance to display their metabolic and immunomodulatory effects than nonadherent strains, (3) they stabilize the intestinal mucosal barrier, and (4) adherence also provides a means of competitive exclusion of pathogenic bacteria from the intestinal epithelium (FAO/WHO, 2002; de Melo Pereira et al., 2018). The gastrointestinal stress that candidate probiotic strains are exposed to during passage along the GIT may act as an intestinal signal that triggers the expression of genes that are related to changes on the bacterial surface. This may affect the adhesion of bacteria to the gut epithelium or the immunomodulatory capacity of candidates (de los Reyes-Gavilan et al., 2011). Such types of interactions with the hosts are demonstrated by bifidobacterial strains and *L. plantarum* WCFS1 (van den Nieuwboer, van Hemert, Claassen, & de Vos, 2016).

The adhesion ability of a candidate depends on various surface determinants. On one side are the passive forces, electrostatic interactions, hydrophobic interactions, steric forces, and lipoteichoic acids, while on the other side are the chemical factors and physiochemical structures such as external appendages covered by lectins (Servin & Coconnier, 2003). In several studies, different adhesion molecules have been described for *Lactobacillus* strains, for example, a protease-resistant and surface-associated bacterial component for *Lb. acidophilus* LB and BG2FO4 (Greene & Klaenhammer, 1994); an adhesion-promoting protein with molecular mass of 29 kDa for *Lb. fermentum* 104R (Rojas, Ascencio, & Conway, 2002); a lipoteichoic acid factor isolated from the cell wall of *Lb. johnsonii* La1 (Granato et al., 1999); and lectin-like protein structures of *Lb. animalis* and *Lb. fermentum* (Gusils, Cuozzo, Sesma, & Gonzalez, 2002). It has been reported that an aggregation-promoting factor (APF) containing 257–326 amino acids were responsible for the adhesion of *Lb. johnsonii* and *Lb. gasseri*, and the encoding genes include two *apf* genes, *apf1* and a*pf2* (Ventura, Jankovic, Walker, Pridmore, & Zink, 2002).

The adhesion ability of a candidate can be assessed by determining the autoaggregation capacity and hydrophobic properties. The high autoaggregation ability of a strain indicates that they can attain a high cell density in the gut, contributing to the adhesion mechanism. On the other hand, improved interaction between microbes and human epithelial cells depends on the high hydrophobicity of bacterial cells (de Melo Pereira et al., 2018). Although the properties of autoaggregation and cell surface hydrophobicity of a candidate can be determined through simple methods, another way to assess microbial adhesion is controlled and comparable in vitro model systems. Mammalian colon carcinoma cell lines, Caco-2, and HT-29, which are differentiated into enterocytes, are widely used as models for the small intestinal epithelium (Servin & Coconnier, 2003; de Melo Pereira et al., 2018).

On the other hand, the use of the Caco-2 and HT-29 cells as an in vitro model for studying adhesion and the mechanisms of action of probiotics may not be useful as they may not always reflect the in vivo adhesion ability of candidates (Vinderola et al., 2017). The primary reasons may be their mixed large- and small-bowel phenotype, different composition of sugars on the cell surface, and cultivation of candidates on surfaces of the plastic monolayers. A study conducted on two commercial probiotic strains LGG and *Lb. casei* Shirota reported that while the strains adhered poorly to Caco-2 cells grown on plastic surfaces, the binding ratio of the same strains to normal intestinal cells was nearly 50% (Cencič & Langerholc, 2010). Another approach that could be useful for selecting the site-specific, function-specific, or disease-specific probiotics is the use of pieces of the human intestinal tissue with local microbiota to mimic conditions in different parts of the intestinal tract (Ouwehand et al., 2002).

3.3 Antipathogenic activity

The competitiveness ability of probiotic candidates, deemed to be appropriate based on their stress tolerance, adhesion, and colonization ability, is related to their antagonism against pathogenic bacteria through the production of antimicrobial substances or competitive exclusion. The second antagonism mechanism mentioned earlier involves competition for attachment to the epithelial surfaces and nutrients, coaggregation with pathogens, and stimulation of the immune system. Although the antimicrobial mechanism varies considerably according to the strain, the antimicrobial components that are produced to defend the host against the invasion of pathogens are mainly organic acids, hydrogen peroxides, and bacteriocins (Gaspar et al., 2018). The coaggregation activity facilitates the agglomeration of pathogens with probiotic cells. This property can be evaluated through a simple method, wherein the absorbance of the coaggregates of probiotics with different pathogens, including *E. coli*, *Staphylococcus aureus*, *Candida* spp., *Listeria monocytogenes*, is measured at various time intervals. The ability of probiotic candidates to produce antimicrobial metabolites has been conventionally evaluated by agar spot (van Belkum, Hayema, Geis, Kok, & Venema, 1989) and well diffusion (Tagg & Mcgiven, 1971) tests in agar plates, where zones of inhibition against indicator microorganisms are determined. With a more innovative approach, the measurement of competitive activity can be determined by the inhibition of adhesion of pathogens to cell lines, which are previously adhered to by the candidate probiotic (de Melo Pereira et al., 2018).

Bacteriocins that are synthesized and secreted in the ribosome are characterized as antimicrobial peptides of length 20–60 amino acids that are cationic, hydrophobic, and capable of inhibiting bacteria causing food spoilage, as well as pathogens. Among the microorganisms that produce bacteriocin, LAB are the most well-known and characterized microbial group. Nisin, produced by distinct strains of *Lactococcus lactis* subsp. *lactis*, can be considered as the most studied bacteriocin among the antimicrobial peptides produced by LAB and has been granted GRAS status for certain applications by the USFDA (Kumariya et al., 2019). Although different mechanisms explain the effects of bacteriocins, another group of bacteriocin (lactococcin A, B, Z, G, and Q), produced by *Lc. lactis* subsp. *lactis*, acts by permeabilizing the cell membrane by recognizing specific regions in the mannose phosphotransferase system of the susceptible cell, or by forming potassium-selective channels in the pathogens (Daba, Ishibashi, Zendo, & Sonomoto, 2017). Pediocin is another antimicrobial peptide that is produced by an important probiotic bacterium, *Pediococcus* spp., which belongs to class II-a bacteriocins and is highly effective against *Listeria* spp. (Uymaz, Şimşek, Akkoç, Ataoğlu, & Akçelik, 2009). Pediocin interacts strongly with the cell wall of the pathogen, enters the cytoplasm, and then disrupts the pathogen's membranes and inhibits protein synthesis by interfering with the replication and transcription of DNA (Khaneghaha et al., 2020). Uymaz Tezel (2019) reported that the novel bacteriocin produced by *Enterococcus lactis* PMD74 has a broad antimicrobial activity, which has been calculated to be 6400 AU/mL.

On the other hand, the wide antimicrobial spectrum of bacteriocins produced by candidate probiotic strains, observed in in vitro assays, may be limited in vivo. The inhibitory effects of traditional bacteriocins are known to be restricted to closely related species such as *Lactobacillus*, or spore-formers such as *Bacillus* or *Clostridium* (Holzapfel, Geisen, & Schillinger, 1995). The other metabolites of probiotic candidates, such as hydrogen peroxide, lactic and acetic acids, and other aromatic compounds, have been reported to show a wide inhibitory spectrum against several harmful organisms such as *Salmonella*, *E. coli*, *Clostridium*, and *Helicobacter* (Saarela et al., 2000; Liu et al., 2009). This phenomenon has been well characterized by the commercial strain LGG. In vitro assays have demonstrated the low molecular weight antimicrobial(s) (possibly short-chain fatty acids) of the strain to have inhibitory activity against anaerobes such as *Clostridium*, *Bacteroides*, and *Bifidobacterium*; against *Enterobacteriaceae*, *Pseudomonas*, *Staphylococcus*, and *Streptococcus* but not against other lactobacilli. On the other hand, in in vivo assays conducted on mice infected with Gram-negative bacteria such as *Salmonella typhimurium*, the antagonistic activity has been shown (Saarela et al., 2000).

4 Safety assessments

The application of probiotic cultures in the food industry requires an assessment of their safety (Holzapfel et al., 1995). Various guidelines explaining the assessment of the safety of probiotics in food are available. The QPS recommendation, which is a valuable tool for controlling the safety assessment of microorganisms in food, was developed by the EFSA. The QPS recommendation for the assessment of probiotic cultures is based on establishing taxonomic identity, the body of knowledge, possible pathogenicity, and commercial end-use (Herman et al., 2019). However, the USFDA has mandated that probiotic cultures should have the GRAS status. Apart from this difference, there are some more differences between EFSA QPS and FDA recommendations of GRAS status. FDA GRAS guidelines are summarized as (1) on the basis of common use, (2) open list, (3) determination of GRAS status by FDA and/or external experts, (4) description of specific substance or microorganisms, and (5) general application to food additives. However, QPS guidelines are listed as (1) on the basis of the history of use and adverse effects; (2) positive list; (3) determination of QPS status by EFSA; (4) description of taxonomic unit (genus, species, or strain); and (5) application to microorganisms only (Jurkovic, Sybesma, Phothirath, Ananta, & Mercenier, 2010).

Based on the guidelines of the FAO/WHO report published in 2002, when choosing a probiotic strain, a multidisciplinary approach is required to examine the pathological, genetic, toxicological, immunological, gastroenterological, and microbiological safety (FAO/WHO, 2002). The minimum requirements of these guidelines needed for conferring probiotic status can be classified into four categories: (1) the assessment of strain identity, (2) in vitro tests, (3) safety assessment, and (4) in vivo studies (Holzapfel et al., 1995). In vitro studies provide the first step in evaluating probiotics for use in food. It is reported that in vitro data of candidate cultures are not sufficient for accepting them as probiotics. In vitro research must be combined with preclinical research and/or full-scaled clinical trials (Foligne, Daniel, & Pot, 2013). In vitro procedures are generally the most preferred, as in vivo methods are expensive and need approval of ethical committees (Nami, Haghshenas, Haghshenas, & Yari, 2015).

Probiotics have also been recommended for both animal and human use. Interestingly, the safety requirements of probiotics in animal foods may be more rigorous than those for human use. Farm animals consuming probiotics will be included in the food chain for human consumption, thus necessitating the safety of probiotics for both animal and human consumption (Gueimonde, Ouwehand, & Salminen, 2004).

4.1 Virulence factors

VFs can determine the pathogenesis of bacterial pathogens. VFs cause infection in both humans and animals. VFs of different strains or species of bacteria can conduct horizontal gene transfer through mobile elements such as plasmids, conjugative transposons, integrons, and/or bacteriophage, making the emergence of new pathotypes of bacteria inevitable (Bennedsen, Stuer-Lauridsen, Danielsen, & Johansen, 2011). Comprehensive characterization of the VFs of probiotic cultures is critical for the effective prevention and control of infectious diseases. Whole-genome sequencing is the most effective method for the determination and complete characterization of the agents of VFs (Liu, Zheng, Jin, Chen, & Yang, 2019). VFs from various bacterial pathogens can be accessed using the virulence factor database (VFDB, http://www.mgc.ac.cn/VFs/). The VFDB contains 1081 VFs, among which 576 are experimentally verified (Anomyous, 2020).

LAB belonging GRAS group contain some potential genes encoding VFs. VFs such as those involved in the production of extracellular proteins (hemolysin and gelatinase), production of enterotoxins or cytotoxins, the capacity to invade host epithelial cells, surface proteins, and aggregation substances can be confirmed by proving the absence of key genes using PCR techniques and/or DNA hybridization. According to VFDB, *Enterococcus* spp. and *Lactobacillus* spp. contained 16 and 1 VFs, respectively (Liu et al., 2019; Anomyous, 2020). *Enterococcus* spp., generally known as nonstarter LAB flora in a variety of traditional foods, have been used in probiotic cultures to a considerably lesser extent. In contrast to other LAB strains, *Enterococcus* does not have a GRAS status and also known to be an opportunistic pathogen for humans and animals. As this genus carries various VFs, its safety for use as probiotic bacteria is of great concern. Major VFs in enterococci are AS (aggregation substance), *Ace* (adhesin to collagen of *Enterococcus faecalis*), *Acm*, *Ebp* (endocarditis- and biofilm-associated pili), *Ecb*A, *Efa*A, *Esp* (enterococcal surface protein), *Scm* (second collagen adhesin of *Enterococcus faecium*), *Sgr*A (serine-glutamate repeat A), *Bop*D (biofilm formation), gelatinase, capsule formation, hyaluronidase, serine protease, *Fsr* (*E. faecalis* regulator), and cytolysin (Şanlıbaba, Uymaz Tezel, & Şentürk, 2018). Baccouri et al. (2019), who aimed to evaluate the probiotic properties of *E. faecalis* OB14 and OB15 strains from traditional artisanal cheeses, reported that the genes *ace* and *esp* were not found in *E. faecalis* OB14 and OB15 strains, whereas genes *asa1* and *gelE* were detected in both strains. Chai et al. (2019) observed that *E. faecalis* 2A (XJ05) carried 18 novel genes of VFs. Wang et al., 2020 reported the prevalence of seven virulence genes in seven *E. faecalis* strains as follows: *asa1* (100%), *cylA* (71.4%), *esp* (85.7%), *hyl* (14.3%), *gelE* (85.7%), *ace* (42.9%), and *agg* (71.4%). Therefore the FAO/WHO Expert Committee (FAO/WHO, 2002) has recommended that *Enterococcus* spp. should not be considered as a probiotic strain for human use.

4.2 Antibiotic resistance

In 1951 the USFDA approved antibiotics as animal feed additives without veterinary prescription. Since then, an increase in the incidence of human infections by antibiotic-resistant bacteria was reported (Hummel, Hertel, Holzapfel, & Franz, 2007). The main cause of the development of antibiotic resistance is the use of antimicrobials in veterinary medicine, due to which antibiotic resistance can easily be transported from animals to humans via the food chain (de Melo Pereira et al., 2018). Antibiotic resistance may be related to chromosomes, transposons, plasmids, insertion sequences, and prophages. There are two types of resistance to antibiotics: intrinsic and acquired. Intrinsic resistance is chromosome related and not transferable to other bacteria (Courvalin, 2006). However, the determinants of acquired resistance can later be transferred to strains of the same species, other species of the same genus, or numerous pathogenic or nonpathogenic bacteria (Economou, Sakkas, Delis, & Gousia, 2017).

The presence of transmissible antibiotic resistance genes in the probiotic strains is a crucial safety criterion. LAB that are frequently used for probiotic cultures often harbor plasmids of different sizes, as well as some antibiotic resistance determinants located on the plasmids (Hummel et al., 2007). So far, *Lactobacillus* species, including *Lb. casei*, *Lb. acidophilus*, *Lb. reuteri*, and *Lb. rhamnosus*, or the yogurt starter bacteria *Lb. delbrueckii*, have only been tested for some antibiotics. Thus the presence of determinants of antibiotic resistance in all the probiotic strains must be systematically screened (Gueimonde, Sanchez, Reyes-Gavilan, & Margolles, 2013). The disk-diffusion method, agar and broth dilution methods for determining of minimum inhibitory concentration of antibiotics, Stokes method, and E-test are routinely used to test the antibiotic resistance of bacteria. The E-test (Epsilometer Test Princip, Ellipse gradient test-AB biodisk) is also preferred (Ashraf & Shah, 2011). Recently, molecular screening has been considered to be a more appropriate tool for determining antibiotic resistance. The antibiotic-resistant genes are characterized using degenerate primers via the 16S rRNA gene sequencing analysis (de Melo Pereira et al., 2018).

Temmerman, Pot, Huys, and Swings (2003) have reported resistance among strains isolated from 55 European probiotic products, against the antibiotic's kanamycin (79%), vancomycin (65%), tetracycline (26%), penicillin G (23%), erythromycin (16%), and chloramphenicol (11%). Moreover, 68.4% of the isolates showed resistance against multiple antibiotics, including intrinsic resistance. Liu et al. (2009) tested 41 strains from commercial probiotic products using disk diffusion

and E-test methods and observed that all the isolates were susceptible to chloramphenicol, tetracycline, ampicillin, amoxicillin/clavulanic acid, cephalothin, and imipenem and resistant to vancomycin, rifampicin, streptomycin, bacitracin, and erythromycin. However, the incidence of resistance to these antibiotics was relatively low. Sharma and Goyal (2015) assessed the antibiotic resistance of five strains from commercial probiotic products called Darolac and Prepro using the Kirby–Bauer disk-diffusion method and reported that almost 80% of the isolates showed resistance to ceftazidime, amoxicillin/clavulanate, and aztreonam.

4.3 Taxonomy/Identification

Potential probiotic cultures can be isolated from a variety of sources such as human samples, soil, dairy products, or the surfaces of vegetables and fruits (McFarland, 2015). Earlier studies have stated that probiotic candidates should be of human origin. This criterion has been suggested on the basis of the assumption that strains of bacterial species that are present in the human intestinal flora could have a better chance of survival in their native environment. On the other hand, studies based on in vitro assays have suggested that bacterial adhesion to epithelial tissues could be "host-specific," for example, lactobacilli isolated from mammals adhere only to mammalian cells (Morelli, 2007). However, FAO/WHO (2001) has suggested that the mandatory requirement of human origin for probiotic bacteria should be reconsidered because of two reasons: (1) the specificity of the action of the microorganism is essential and not the source and (2) it is impossible to identify the source of an organism (Morelli, 2007). In this context, it is important to emphasize that the probiotic effect is strain specific (Figueroa-Gonzalez, Quijano, Ramirez, & Cruz-Guerroro, 2011). Therefore probiotic strains must first be accurately identified and characterized up to their genus, species, and strain level (Fijan, 2014). According to the guidelines of the World Gastroenterology Organization Global Guidelines, a probiotic strain is also named alphanumerically. In the global scientific community, an agreed nomenclature for microorganisms has been accepted (e.g., *Lb. casei* DN-114 or LGG) (World Gastroenterology Organization, 2011). Classical microbiological tests remain crucial for culture propagation, selection, enumeration, and phenotypic characterization. However, these properties are not sufficient for the identification of closely related species, such as the *Lb. acidophilus*, which has similar ecological niches and is likely to play similar functional roles. Moreover, molecular tools offer the most accurate analysis for the taxonomic classification of bacterial cultures (Klaenhammer & Kullen, 1999; Gueimonde et al., 2004). Novel molecular technologies can be used for the identification of the specific strain. Analysis of the 16S/23S rRNA gene and DNA–DNA hybridization techniques can be counted among such techniques. However, analysis of the 16S rRNA gene is not always reliable, such as in the *Lb. casei* group, due to high sequence similarities (Hill et al., 2018). In such cases, other reproducible molecular techniques, such as pulsed-field gel electrophoresis; randomly amplified polymorphic DNA analysis, including multiplex PCR, arbitrary primed PCR, and triplet arbitrary primed PCR; ribotyping; restriction enzyme analysis; multilocus sequence typing; repetitive element palindromic PCR; and denaturing gradient gel electrophoresis may be used (Fijan, 2014; de Melo Pereira et al., 2018; Hill et al., 2018). Recently, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry has been used for the identification and classification of bacterial isolates according to their protein profiles (Foligne et al., 2013). The presence of extrachromosomal genetic tools such as plasmids can also contribute to both typing and characterization of the strain (Hill et al., 2018).

5 Technological requirements

The safety of food products, including novel probiotic cultures, should be evaluated before adding to the products. There may be several factors to affect them, such as the historical scientific reports of their laboratory assay and their history of safe use in humans, as well as consumer satisfaction (Peivasteh-Roudsari et al., 2019). Among these factors, several desirable criteria about technological requirements in different types of food matrices have been recommended for the selection of suitable probiotic microorganisms for commercial application (Samedi & Charles, 2019). These criteria can be summarized as (1) genetically stable strains, (2) desired viability during processing and storage (at least 10^6 CFU/g or CFU/mL of viable cells), (3) good sensory properties, (4) large-scale production, (5) phage resistance, (6) genetic flexibility, (7) stability of desired characteristics during culture preparation, and (8) anticarcinogenic properties (Mattila-Sandholma et al., 2002). Parameters of food, processing, and microbiology are important factors that affect the viability of probiotic cultures during the production of food. Food parameters include pH, titratable acidity, molecular oxygen content, water activity, presence of salt, sugar, and chemicals (hydrogen peroxide, food ingredients, and food additives such as sugars, salt, antimicrobials, aromatic compounds, or even bacteriocins) (Terpou et al., 2019). Processing parameters, including fermentation conditions, involve heat treatment, incubation temperature, the cooling rate of food, packaging aspects, methods of storage, storage temperature, and scale of the probiotic food production. Finally, the strain type of probiotics, antagonism

with starter cultures, occurrence of several pathogenic and/or spoilage microorganisms, produced enzymes by microorganisms, and proportion of inoculation are the microbiological parameters (Tripathi & Giri, 2014).

As mentioned before, the probiotic strains must be resistant to the gastric acid conditions of low pH in the stomach. Potential probiotic strains should also not destroy bile salts via hydroxylation or decongestion in the small intestine (Peivasteh-Roudsari et al., 2019). Moreover, these strains must survive in the stress conditions such as the high concentration of bile salts in the small intestine, pass the GIT, and colonize the gut epithelial tissue of the host. Consequently, they may interact with other bacteria and pathogens (Gupta, Jeevaratnam, & Fatima, 2018). Other technological selection criteria of probiotic strains for the assessment of safety include (1) production of organic acid; (2) increase of metabolic activities; (3) the absence of β-hemolytic activity; (4) degradation of mucins that are high molecular mass glycoproteins secreted by the goblet cells; (5) inability to produce biogenic amines; (6) antimutagenicity; (7) reduction in lactose intolerance; and (8) the absence of harmful enzymes such as β-glucosidase, *N*-acetyl-β-glucosaminidase, and β-glucuronidase (the presence of all enzymatic activities connected with health disorders or intestinal diseases) (Roy, 2005; Wedajo, 2015).

There are also several technological requirements of potential probiotic strains for use in the food systems (Espitia, Batista, Azeredo, & Otoni, 2016). First, the acidifying ability of probiotic cultures is one of the most desirable metabolic properties of LAB used in the food industry. It can occur due to the production of lactic acid from the fermentation of carbohydrates during the growth of the probiotic bacteria. The production of lactic acid during fermentation has several benefits: (1) lowering of pH of both culture media and food matrices; (2) decrease in the risk of infection by pathogenic flora such as *Helicobacter pylori*, *Salmonella* spp., *L. monocytogenes*, and *Clostridium difficile*; and (3) change in the final products (Kosin & Rakshit, 2006; Samedi & Charles, 2019). The potential probiotic strains might grow slowly in low pH conditions and may also give off-flavors (Mattila-Sandholma et al., 2002). This problem could be partly overcome by producing probiotic-based foods with levels of probiotic cultures reaching approximately 10^9 CFU/g or CFU/mL. Another approach to overcome this problem could be the addition of supplementary energy sources, growth factors, or suitable antioxidants, minerals, and vitamins during the fermentation process to improve the suitability of the food as a substrate for the probiotic strains. Particular attention should be paid to the acid-forming ability for selecting probiotic strains, as their properties could be connected to the effect on human health (Saarela et al., 2000). Second, proteolytic and lipolytic capacities are connected with probiotic culture species. LAB demonstrate different potentialities based on their enzymatic equipment for the use of the nitrogen fraction. In general, lactobacilli are more proteolytic than lactococci. LAB strains generally show lower lipolytic characteristics. Moreover, lactococci are considered to be more lipolytic than *Strep. thermophilus* and lactobacilli. In the production of probiotic cheese, both proteolytic and lipolytic properties are essential (Peirotén, Gaya, Arqués, Medina, & Rodríguez, 2019; Samedi & Charles, 2019). Furthermore, LAB can produce several aromatic compounds, such as α-acetolactate, acetaldehyde, diacetyl, acetoin, 2,3-butanediol, ethanol, and acetate, from lactose, citrate, amino acids, and fats. The flavoring ability of probiotic cultures is particularly important in the production of fermented probiotic products such as fresh cheeses, creams, and butter; hence, their primary aroma is based on microbial activity (Peivasteh-Roudsari et al., 2019; Samedi & Charles, 2019). Moreover, LAB can synthesize exopolysaccharides (EPS) that play a vital role in the consistency and rheology of processed products. EPS-producing *Lb. delbrueckii* subsp. *bulgaricus* and *Strep. thermophilus* are commonly used in probiotic yogurts (Wedajo, 2015). Finally, LAB produce numerous antimicrobial compounds that are used in the bioconservation of food. This activity is one of the most important selection criteria for probiotic cultures (Nader-Macías & Tomás, 2015; Samedi & Charles, 2019). Antimicrobial effects of LAB are a result of the production of many substances such as organic acids (lactic, acetic, propionic acids, and diacetyl), carbon dioxide, hydrogen peroxide, low molecular weight antimicrobial substances, and bacteriocins (Klaenhammer & Kullen, 1999). The antimicrobials that are produced by LAB show excellent antibacterial, antifungal, and antiviral activities against harmful pathogens. Among these antimicrobials, the production of bacteriocin by probiotic strains is a particularly important factor during the processing of food. Although probiotic strains are able to produce bacteriocins, limited inhibitory activity against pathogens is observed in in vivo assays (Saarela et al., 2000). In general, the inhibitory activity of bacteriocins is directed against species that are closely related to the producing strain, and their spectrum of action is generally narrow (Gupta et al., 2018). However, low molecular weight metabolites and secondary metabolites produced by probiotic strains may be more important, because they show a wide inhibitory spectrum against several harmful organisms (Saarela et al., 2000).

6 Conclusion

The studies on probiotic microorganisms, their selection criteria, and assessment of their safety in humans and animals are receiving attention throughout the world. Considerable effort is spent on the selection criteria of probiotic cultures. The connection between selection and safety criteria for probiotic cultures can be based on their microbiology. Numerous

criteria have been suggested for screening and selecting suitable probiotic microorganisms. Technological, functional, physiological, and safety criteria should be investigated for the selection of probiotics in commercial food applications. Fulfillment of these criteria makes the probiotic cultures suitable for large-scale food production.

References

Abdel-Rahman, M. A., Tashiro, Y., & Sonomoto, K. (2013). Recent advances in lactic acid production by microbial fermentation processes. *Biotechnology Advances*, *31*, 877–902.

Anomyous, (2020). https://www.mgc.ac.cn/VFs/status.htm Access Date: 21.04.2020.

Ashraf, R., & Shah, N. P. (2011). Antibiotic resistance of probiotic organisms and safety of probiotic dairy products. *International Food Research Journal*, *18*(3), 837–853.

Baccouri, O., Boukerb, A. M., Farhat, L. B., Zébré, A., Zimmermann, K., Domann, E., et al. (2019). Probiotic potential and safety evaluation of *Enterococcus faecalis* OB14 and OB15, isolated from traditional Tunisian testouri cheese and rigouta, using physiological and genomic analysis. *Frontiers in Microbiology*, *10*, 881.

Bennedsen, M., Stuer-Lauridsen, B., Danielsen, M., & Johansen, E. (2011). Screening for antimicrobial resistance genes and virulence factors via genome sequencing. *Applied and Environmental Microbiology*, *77*(8), 2785–2787.

Blanquet, S., Zeijdner, E., Beyssac, E., Meunier, J. P., Denis, S., Havenaar, R., et al. (2004). A dynamic artificial gastrointestinal system for studying the behaviour of orally administered drug dosage forms under various physiological conditions. *Pharmaceutical Research*, *21*, 585–591.

Cencič, A., & Langerholc, T. (2010). Functional cell models of the gut and their applications in food microbiology – A review. *International Journal of Food Microbiology*, *14*, S4–S14.

Chai, Y., Gu, X., Wu, Q., Gua, B., Qi, Y., Wang, X., et al. (2019). Genome sequence analysis reveals potential for virulence genes and multidrug resistance in an *Enterococcus faecalis* 2A (XJ05) strain that causes lamb encephalitis. *BMC Veterinary Research*, *15*, 235.

Corcoran, B. M., Stanton, C., Fitzgerald, G. F., & Ross, R. P. (2005). Survival of probiotic *Lactobacilli* in acidic environments is enhance in the presence of metabolizable sugars. *Applied and Environmental Microbiology*, *71*(6), 3060–3067.

Cotter, P. D., & Hill, C. (2003). Surviving the acid test: Responses of gram-positive bacteria to low pH. *Microbiology and Molecular Biology Reviews*, *67*, 429–453.

Courvalin, P. (2006). Antibiotic resistance: The pros and cons of probiotics. *Digestive and Liver Disease*, *38*(2), 261–265.

Daba, G. M., Ishibashi, N., Zendo, T., & Sonomoto, K. (2017). Functional analysis of the biosynthetic gene cluster required for immunity and secretion of a novel *Lactococcus*-specific bacteriocin, lactococcin Z. *Journal of Applied Microbiology*, *123*, 1124–1132.

de los Reyes-Gavilan, C. G., Suarez, A., Fernandez-Garcia, M., Margolles, A., Gueimonde, M., & Ruas-Madiedo, P. (2011). Adhesion of bile-adapted *Bifidobacterium* strains to HT29-MTX cell line is modified after sequential gastrointestinal challenge simulated *in vitro* using human gastric and duodenal juices. *Research in Microbiology*, *162*, 514–519.

de Melo Pereira, G. V., de Oliveira Coelho, B., Júnior, A. I. M., Thomaz-Soccol, V., & Soccol, C. R. (2018). How to select a probiotic? A review and update of methods and criteria. *Biotechnology Advances*, *36*, 2060–2076.

Divya, J. B., Varsha, K. K., & Nampoothiri, K. M. (2012). Probiotic fermented foods for health benefits. *Engineering in Life Sciences*, *12*(4), 377–390. doi: 10.1002/elsc.201100179.

Economou, V., Sakkas, H., Delis, G., & Gousia, P. (2017). Chapter 16: Antibiotic resistance in *Enterococcus* spp. friend or foe? In O. V. Singh (Ed.), *Foodborne pathogens and antibiotic resistance* (pp. 365–395). Hoboken, New Jersey: John Wiley&Sons.

Espitia, P. J. P., Batista, R. A., Azeredo, H. M. C., & Otoni, C. G. (2016). Probiotics and their potential applications in active edible films and coatings. *Food Research International*, *90*, 42–52.

FAO/WHO. (2001). *Health and nutrition properties of probiotics in food including powder milk with live lactic acid bacteria* (pp. 1–34). Rome, Italy: FAO/WHO. https://isappscience.org/wp-content/uploads/2015/12/FAO-WHO-2001-Probiotics.

FAO/WHO. (2002). Guidelines for the evaluation of probiotics in food. *Report of a joint FAO/WHO Working Group on drafting guidelines for the evaluation of probiotics in food* (pp. 1–11). London, Ontario, Canada: World Health Organization.

Figueroa-Gonzalez, I., Quijano, G., Ramirez, G., & Cruz-Guerroro, A. (2011). Probiotics and prebiotics-perspectives and challenges. *Journal of the Science of Food and Agriculture*, *91*, 1341–1348.

Fijan, S. (2014). Microorganisms with claimed probiotic properties: An overview of recent literature. *International Journal of Environmental Research and Public Health*, *11*, 4745–4767.

Floch, M. H. (2013). Probiotic safety and risk factors. *Journal of Clinical Gastroenterology*, *47*(5), 375–376.

Foligne, B., Daniel, C., & Pot, B. (2013). Probiotics from research to market: The possibilities, risks and challenges. *Current Opinion in Microbiology*, *16*, 284–292.

Fuller, R. (1989). A review: Probiotics in man and animals. *Journal of Applied Bacteriology*, *66*, 365–378.

Gaspar, C., Donders, G. C., Palmeira-de-Oliveira, R., Queiroz, J. A., Tomaz, C., Martinezde-Oliveira, J., et al. (2018). Bacteriocin production of the probiotic *Lactobacillus acidophilus* KS400. *AMB Express*, *8*(1), 153.

Gil-Rodríguez, A. M., Carrascosa, A. V., & Requena, T. (2015). Yeasts in foods and beverages: In vitro characterisation of probiotic traits. *LWT—Food Science and Technology*, *64*, 1156–1162.

Gorbach, S., Doron, S., & Magro, F. (2017). *Lactobacillus rhamnosus* GG. In M. H. Floch, Y. Ringel, & W. A. Walker (Eds.), *The microbiota in gastrointestinal pathophysiology* (pp. 79–88). Amsterdam, the Netherlands: Elsevier.

Granato, D., Perotti, F., Masserey, I., Rouvet, M., Golliard, M., Servin, A., et al. (1999). Cell surface-associated lipoteichoic acid acts as an adhesion factor for attachment of *Lactobacillus johnsonii* La1 to human enterocyte-like Caco-2 cells. *Applied and Environmental Microbiology*, *65*, 1071–1077.

Greene, J. D., & Klaenhammer, T. R. (1994). Factors involved in adherence of lactobacilli to human Caco-2 cells. *Applied and Environmental Microbiology*, *60*, 4487–4494.

Gueimonde, M., Ouwehand, A. C., & Salminen, S. (2004). Safety of probiotics. *Scandinavian Journal of Nutrition*, *48*(1), 42–48.

Gueimonde, M., Sanchez, B., Reyes-Gavilan, C. G., & Margolles, A. (2013). Antibiotic resistance in probiotic bacteria. *Frontiers in Microbiology*, *4*, 1–6.

Gupta, R., Jeevaratnam, K., & Fatima, Y. A. (2018). Lactic acid bacteria: Probiotic characteristic, selection criteria, and its role in human health (a review). *International Journal of Emerging Technologies and Innovative Research*, *5*(10), 411–424.

Gusils, C., Cuozzo, S., Sesma, F., & Gonzalez, S. (2002). Examination of adhesive determinants in three species of *Lactobacillus* isolated from chicken. *Canadian Journal of Microbiology*, *48*, 34–42.

Herman, L., Chemaly, M., Cocconcelli, P. S., Fernandez, P., Klein, G., Peixel, L., et al. (2019). The qualified presumption of safety assessment and its role in EFSA risk evaluations: 15 years past. *FEMS Microbiology Letters*, *366*, fny260.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., et al. (2014). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*, *11*(8), 506–514.

Hill, D., Sugrue, I., Tobin, C., Hill, C., Stanton, C., & Ross, R. P. (2018). The *Lactobacillus casei* Group: History and health related applications. *Frontiers in Microbiology*, *9*, 2107.

Holzapfel, W. H., Geisen, R., & Schillinger, G. U. (1995). Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *International Journal of Food Microbiology*, *24*, 343–362.

Hummel, A. S., Hertel, C., Holzapfel, W. H., & Franz, C. M. A. P. (2007). Antibiotic resistances of starter and probiotic strains of lactic acid bacteria. *Applied and Environmental Microbiology*, *73*(3), 730–739.

Jacobsen, C. N., Rosenfeldt, V., Nielsen, A., Hayford, E., Moller, P. L., Michaelsen, K. F., et al. (1999). Screening of probiotic activities of forty-seven strains of *Lactobacillus* spp. by *in vitro* techniques and evaluation of the colonization ability of five selected strains in humans. *Applied Environmental Microbiology*, *65*, 4949–4956.

Jurkovic, I., Sybesma, W., Phothirath, P., Ananta, E., & Mercenier, A. (2010). Application of probiotics in food products-challenges and new approaches. *Current Opinion in Biotechnology*, *21*, 175–181.

Khaneghaha, A. M., Abharib, K., Eşc, I., Soaresa, M. B., Oliveira, R. B. A., Hosseini, H., et al. (2020). Interactions between probiotics and pathogenic microorganisms in hosts and foods: A review. *Trends in Food Science & Technology*, *95*, 205–218.

Klaenhammer, T. R., & Kullen, M. J. (1999). Selection and design of probiotics. *International Journal of Food Microbiology*, *50*, 45–57.

König, H., & Fröhlich, J. (2009). Lactic acid bacteria. In H. König, G. Unden, & J. Fröhlich (Eds.), *Biology of microorganisms on grapes, in must and in wine* (pp. 3–29). Berlin, Heidelberg: Springer-Verlag.

Kosin, B., & Rakshit, S. K. (2006). Microbial and processing criteria for production of probiotics: A review. *Food Technology and Biotechnology*, *44*(3), 371–379.

Kumariya, R., Garsa, A. K., Rajput, Y. S., Sood, S. K., Akhtar, N., & Patel, S. (2019). Bacteriocins: Classification, synthesis, mechanism of action and resistance development in food spoilage causing bacteria. *Microbial Pathogenesis*, *128*, 171–177.

Lee, J. H., & O'Sullivan, D. J. (2010). Genomic insights into *Bifidobacteria*. *Microbiology and Molecular Biology Reviews*, *74*, 378–416.

Liu, C., Zhang, Z. Y., Dong, K., Yuan, J. P., & Guop, X. K. (2009). Antibiotic resistance of probiotic strains of lactic acid bacteria isolated from marketed foods and drugs. *Biomedical and Environmental Sciences*, *22*, 401–412.

Liu, B., Zheng, D., Jin, Q., Chen, L., & Yang, J. A. (2019). VFDB 2019: A comparative pathogenomic platform with an interactive web interface. *Nucleic Acids Research*, *47*(Database issue), D687–D692.

Martinez, R. C., Aynaou, A. E., Albrecht, S., Schols, H. A., De Martinis, E. C., Zoetendal, E. G., et al. (2011). *In vitro* evaluation of gastrointestinal survival of *Lactobacillus amylovorus* DSM 16698 alone and combined with galactooligosaccharides, milk and/or *Bifidobacterium animalis* subsp. *lactis* Bb-12. *International Journal Food Microbiology*, *149*, 152–158.

Mathieu, M., Nguyen, A., Amine, K. M., & Lacroix, M. (2013). Intestinal survival of bacteria in commercial probiotic products. *International Journal of Probiotics and Prebiotics*, *8*(4), 149–156.

Mattila-Sandholma, T., Myllarinen, P., Crittendena, R., Mogensen, G., Fonden, R., & Saarelaa, M. (2002). Technological challenges for future probiotic foods. *International Dairy Journal*, *12*, 173–182.

McFarland, L. V. (2015). From yaks to yoghurt: The history, development, and current use of probiotics. *Clinical Infectious Diseases*, *60*(2), 85–90.

Melo, T. A., Santos, T. F. D., Pereira, L. R., Passos, H. M., Rezende, R. P., & Romano, C. C. (2017). Functional profile evaluation of *Lactobacillus fermentum* TCUESC01: A new potential probiotic strain isolated during cocoa fermentation. *Biomed Research International*, *2017*, 1–7. 5165916.

Morelli, L. (2007). *In vitro* assessment of probiotic bacteria: From survival to functionality. *International Dairy Journal*, *17*, 1278–1283.

Nader-Macías, E. F. M., & Tomás, M. S. J. (2015). Profiles and technological requirements of urogenital probiotics. *Advances Drug Delivery Reviews*, *92*, 84–104.

Nami, Y., Haghshenas, B., Haghshenas, M., & Yari, A. (2015). Antimicrobial activity and the presence of virulence factors and bacteriocin structural genes in *Enterococcus faecium* CM33 isolated from ewe colostrum. *Frontiers in Microbiology*, *6*, 782.

Neish, A. S. (2017). Probiotics of the acidophilus group: *Lactobacillus acidophilus*, *delbrueckii* subsp. *bulgaricus* and *johnsonii*. In M. H. Floch, Y. Ringel, & W. A. Walker (Eds.), *The microbiota in gastrointestinal pathophysiology* (pp. 71–78). Amsterdam, the Netherlands: Elsevier.

Ouwehand, A. C., Salminen, S., Tölkkö, S., Roberts, P., Ovaska, J., & Salminen, E. (2002). Resected human colonic tissue: New model for characterizing adhesion of lactic acid bacteria. *Clinical and Diagnostic Laboratory Immunology*, *9*, 184–186.

Peirotén, A., Gaya, P., Arqués, J. L., Medina, M., & Rodríguez, E. (2019). Technological properties of bifidobacterial strains shared by mother and child. *Hindawi Food Microbiology*, *2019*, 1–8, 9814623.

Peivasteh-Roudsari, L., Pirhadi, M., Karami, H., Tajdar-Oranj, B., Molaee-Aghaee, E., & Sadighara, P. (2019). Probiotics and food safety: An evidence-based review. *Journal of Food Safety and Hygiene*, *5*(1), 1–9.

Pradhan, D., Mallappa, R. H., & Grover, S. (2020). Comprehensive approaches for assessing the safety of probiotic bacteria. *Food Control*, *108*, 1–14.

Quigley, E. M. M. (2017a). *Bifidobacterium animalis* spp. *lactis*. In M. H. Floch, Y. Ringel, & W. A. Walker (Eds.), *The microbiota in gastrointestinal pathophysiology* (pp. 127–141). Amsterdam, the Netherlands: Elsevier.

Quigley, E. M. M. (2017b). *Bifidobacteria* as probiotic organisms: An introduction. In M. H. Floch, Y. Ringel, & W. A. Walker (Eds.), *The microbiota in gastrointestinal pathophysiology* (pp. 125–126). Amsterdam, the Netherlands: Elsevier.

Ricci, A., Allende, A., Bolton, D., Chemaly, M., Davies, R., Girones, R. R., et al. (2017). Update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 5: Suitability of taxonomic units notified to EFSA until September 2016. *EFSA Journal*, *15*(3), 1–42.

Rojas, M., Ascencio, F., & Conway, P. L. (2002). Purification and characterization of a surface protein from *Lactobacillus fermentum* 104R that binds to porcine small intestinal mucus and gastric mucin. *Applied and Environmental Microbiology*, *68*, 2330–2336.

Ronka, E., Malinen, E., Saarela, M., Rinta-Koski, M., Aarnikunnas, J., & Palva, A. (2003). Probiotic and milk technological properties of *Lactobacillus brevis*. *International Journal of Food Microbiology*, *83*, 63–74.

Roy, D. (2005). Technological aspects related to the use of bifidobacteria in dairy products. *Le Lait*, *85*(1–2), 39–56.

Ruiz-Moyano, S., Tao, N., Underwood, M. A., & Mills, D. A. (2012). Rapid discrimination of *Bifidobacterium animalis* subspecies by matrix-assisted laser desorption ionization time of flight mass spectrometry. *Food Microbiology*, *30*(2), 432–437.

Russell, D. A., Ross, R. P., Fitzgerald, G. F., & Stanton, C. (2011). Metabolic activities and probiotic potential of *Bifidobacteria*. *International Journal of Food Microbiology*, *149*, 88–105.

Saarela, M., Mogensen, G., Fondén, R., Mättö, J., & Mattila-Sandholm, T. (2000). Probiotic bacteria: Safety, functional and technological properties. *Journal of Biotechnology*, *84*, 197–215.

Samedi, L., & Charles, A. L. (2019). Evaluation of technological and probiotic abilities of local lactic acid bacteria. *Journal of Applied & Environmental Microbiology*, *7*(1), 9–19.

Şanlıbaba, P., Uymaz Tezel, B., & Şentürk, E. (2018). Antimicrobial resistance of *Enterococcus* species isolated from chicken in Turkey. *Korean Journal for Food Science of Animal Resources*, *38*(2), 391–402.

Schultz, M., Burton, J. P., & Chanyi, R. M. (2017). Use of *Bacillus* in human intestinal probiotic applications. In M. H. Floch, Y. Ringel, & W. A. Walker (Eds.), *The microbiota in gastrointestinal pathophysiology* (pp. 119–123). Amsterdam, the Netherlands: Elsevier.

Scott, K. A., Ida, M., Peterson, V. L., Prenderville, J. A., Moloney, G. M., Izumo, T., et al. (2017). Revisiting Metchnikoff: Age-related alterations in microbiota-gut-brain axis in the mouse. *Brain, Behaviour, and Immunity*, *65*, 20–32.

Sebald, W., Friedl, P., Schairer, H. U., & Hoppe, J. (1982). Structure and genetics of the H^+-conducting F0 portion of the ATP synthase. *Annals of the New York Academy of Science*, *402*, 28–44.

Segers, M., & Lebeer, S. (2014). Towards a better understanding of *Lactobacillus rhamnosus* GG–host interactions. *Microbial Cell Factories*, *13*, S7.

Sena, S., & Mansella, T. J. (2020). Yeasts as probiotics: Mechanisms, outcomes, and future potential. *Fungal Genetics and Biology*, *137*, 103333.

Servin, A. L., & Coconnier, M. H. (2003). Adhesion of probiotic strains to the intestinal mucosa and interaction with pathogens. *Best Practice & Research Clinical Gastroenterology*, *17*(5), 741–754.

Sharma, J., & Goyal, A. (2015). A Study on the drug resistance of probiotic strains isolated from commercial probiotic products available in the local market of Agra. *European Journal of Experimental Biology*, *5*(2), 33–36.

Sumeri, I., Arike, L., Adamberg, K., & Paalme, T. (2008). Single bioreactor gastrointestinal tract simulator for study of survival of probiotic bacteria. *Applied Microbiology and Biotechnology*, *80*, 317–324.

Sumi, C. D., Yang, B. W., Yeo, I. C., & Hahm, Y. T. (2015). Antimicrobial peptides of the genus *Bacillus*: A new era for antibiotics. *Canadian Journal of Microbiology*, *61*(2), 93–103.

Tagg, J. R., & Mcgiven, A. R. (1971). Assay systems for bacteriocins. *Applied Environmental Microbiology*, *21*, 943–947.

Temmerman, R., Pot, B., Huys, G., & Swings, J. (2003). Identification and antibiotic susceptibility of bacterial isolates from probiotic products. *International Journal of Food Microbiology*, *81*(1), 1–10.

Terpou, A., Papadaki, A., Lappa, I. K., Kachrimanidou, V., Bosnea, L. A., & Kopsahelis, N. (2019). Probiotics in food systems: Significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. *Nutrients*, *11*, 1591–1599.

Tripathi, M. K., & Giri, S. K. (2014). Probiotic functional foods: Survival of probiotics during processing and storage. *Journal of Functional Foods*, *9*, 225–241.

Uymaz, B., Şimşek, Ö., Akkoç, N., Ataoğlu, H., & Akçelik, M. (2009). *In vitro* characterization of probiotic properties of *Pediococcus pentosaceus* BH105 isolated from human faeces. *Annals of Microbiology*, *59*(3), 485–491.

Uymaz Tezel, B. (2019). Preliminary *in vitro* evaluation of the probiotic potential of the bacteriocinogenic strain *Enterococcus lactis* PMD74 isolated from ezine cheese. *Hindawi Journal of Food Quality*, *2019*, 1–12, 4693513.

van Belkum, M. J., Hayema, B. J., Geis, A., Kok, J., & Venema, G. (1989). Cloning of two bacteriocin genes from a lactococcal bacteriocin plasmid. *Applied Environmental Microbiology*, *55*, 1187–1191.

van den Nieuwboer, M., van Hemert, S., Claassen, E., & de Vos, W. M. (2016). *Lactobacillus plantarum* WCFS1 and its host interaction: A dozen years after the genome. *Microbiology and Biotechnology*, *9*, 452–465.

Ventura, M., Jankovic, I., Walker, D. C., Pridmore, R. D., & Zink, R. (2002). Identification and characterization of novel surface proteins in *Lactobacillus johnsonii* and *Lactobacillus gasseri*. *Applied and Environmental Microbiology*, *68*, 6172–6181.

Vinderola, G., Gueimonde, M., Gomez-Gallego, C., Delfederico, L., & Salminen, S. J. (2017). Correlation between *in vitro* and *in vivo* assays in selection of probiotics from traditional species of bacteria. *Trends in Food Science and Technology*, *68*, 83–90.

Vinderola, G., Reinheimer, J., & Salminen, S. (2019). The enumeration of probiotic issues: From unavailable standardised culture media to a recommended procedure? *International Dairy Journal*, *96*, 58–65.

Wang, J., Da, R., Tuo, X., Cheng, Y., Wei, J., Jiang, K., et al. (2020). Probiotic and safety properties screening of *Enterococcus faecalis* from healthy Chinese infants. *Probiotics and Antimicrobial Proteins*, *12*, 1115–1125. doi: 10.1007/s12602-019-09625-7.

Wedajo, B. (2015). Lactic acid bacteria: Benefits, selection criteria and probiotic potential in fermented food. *Journal of Probiotics & Health*, *3*, 129.

World Gastroenterology Organization. (2011). Probiotics and prebiotics. *World Gastroenterology Organization Global Guidelines: probiotics and prebiotics October 2011* (pp. 1–35). Milwaukee: World Gastroenterology Organization.

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Chapter 3

Simulated Gastrointestinal System to Assess the Probiotic Properties Modified to Encapsulation of Probiotics and Their Survival Under Simulated Gastrointestinal System

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1 Introduction

The term "probiotic" is derived from Greek, meaning "for life" (Fuller, 1989) and includes a large range of microorganisms, mainly bacteria but also yeasts. As more and more light was thrown on the understanding of the mechanisms of probiotic action and their effects on human health unfolded, an evolution of the definition occurred. A probiotic is a live microbial feed supplement that beneficially affects the host by improving its intestinal microbial balance. Later, probiotics are defined as "live micro-organisms which when administered in adequate amounts confer a health benefit on the host" (Reid et al., 2003). These definitions imply that probiotic products, for example, probiotic yoghurts and drinks, contain live microorganisms and improve the health status of the host by exerting beneficial effects on the gastrointestinal tract (GIT). The potential health benefits of probiotic bacteria (*Lactobacillus acidophilus* and *Bifidobacteria*) are enhancement of immunity against intestinal infections, immune enhancement, prevention of diarrheal diseases, prevention of colon cancer, prevention of hypercholesterolemia, improvement in lactose utilization, prevention of upper gastrointestinal (GI) intestinal tract diseases, and stabilization of the gut mucosal barrier (Kailasapathy & Chin, 2000). The use of probiotic cultures stimulates the growth of preferred microorganisms, crowds out potentially harmful bacteria, and reinforces the body's natural defense variety of ways to resist such a wide range of infection agents. The mechanisms of antipathogenic effects may be through decreasing the luminal pH by the production of organic acids (acetic, lactic, or propionic acids), rendering vital nutrients unavailable to pathogens, changing the redox potential of intestinal environment, producing bacteriocins, competing with pathogens for the receptor sites on the intestinal wall (competitive exclusion), or producing hydrogen peroxide, antimicrobial, or other inhibitory substances (Kailasapathy & Chin, 2000). The activities of probiotics may include cell-mediated immune responses, including activation of the reticuloendothelial system, augmentation of cytokine pathways, and stimulation of proinflammatory pathways, such as interleukin regulation (Isolauri, Arvola, Sutas, Moilanen, & Salminen, 2000a; Gill, Cross, Rutherfurd, & Gopal, 2001; Paturi, 2007). These therapeutic properties of probiotic bacteria have been used earlier for various treatments such as lactose intolerance, acute gastroenteritis, food allergy, atopic dermatitis, Crohn's disease, arthritis, and colon cancer (Kalliomaki, Salminen, Poussa, Arvilommi, & Isolauri, 2003; Paturi, 2007) (Hughes & Hoover, 1991; Kailasapathy & Chin, 2000). The activities of probiotics may include cell-mediated immune responses, including activation of the reticuloendothelial system, augmentation of cytokine pathways, and stimulation of proinflammatory pathways, such as interleukin regulation (Isolauri, Arvola, Sutas, Moilanen, & Salminen, 2000b; Gill et al., 2001; Paturi, 2007). These therapeutic properties of probiotic bacteria are proposed for various treatments of human intestinal barrier abnormalities, such as lactose intolerance, acute gastroenteritis, food allergy, atopic dermatitis, Crohn's disease, arthritis, and colon cancer (Kalliomaki et al., 2003; Rinkinen, Jalara, Westermark, Salminen, & Ouwehand, 2003; Paturi,

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00003-4
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2007). Moreover, there are various factors have been reported to affect the viability of probiotics in fermented dairy products, including increased concentration of organic acids, low pH, hydrogen peroxide, dissolved oxygen content, storage temperature, and postacidification during storage of fermented products, such as yoghurt, species and strains of associative, fermented dairy product organisms, and buffering capacity of the ingredients, such as whey proteins concentration (Dave & Shah, 1997). Therefore there is a need to protect the probiotic bacteria against adverse environments, processing, and storage conditions as well as during transit in the GIT. Different approaches that increase the resistance of these sensitive organisms against adverse conditions have been proposed, including appropriate selection of acid- and bile-resistant strains, control of over- and postfermentation acidification of dairy products, addition of cysteine, or an oxygen scavenger, such as ascorbic acid, a two-step fermentation, addition of buffering agents, such as whey protein concentrates, use of oxygen-impermeable or oxygen-scavenging packaging containers, stress adaptation, and incorporation of micronutrients, such as peptides, and amino acids (Kailasapathy & Supriadi, 1996; Dave & Shah, 1997; Krasaekoopt, Bhandari, & Deeth, 2003a). Encapsulation is recently gaining considerable attention to provide the required protection for probiotic bacteria (Kailasapathy & Sultana, 2003; Krasaekoopt, Bhandari, & Deeth, 2003b). Encapsulation is a physicochemical or mechanical inclusion or immobilization technique for confining particles containing active ingredients in a polymeric matrix, coated by one or more semipermeable polymers, by virtue of which the encapsulated compound becomes more stable than its isolated or free form, thus providing protection. Controlled release of the encapsulated active ingredients under specific conditions is a critical benefit of microencapsulation. A microcapsule consists of a semipermeable, spherical, thin, and strong membrane surrounding a solid/liquid core, with a diameter varying from a few micrometers to 1 mm.

2 The gastrointestinal (GI) tract

2.1 Microbiota of the adult GI tract

Complex array of microbiota are present in the adult GI tract with the dominant microbial population of the species of *Bacteroides*, *Eubacterium*, *Clostridium*, and *Peptostreptococcus.* The subdominant populations of bacteria include *Escherichia coli* and streptococci. These are present within the colon as a consequence of the environment produced by the strictly anaerobic bacteria (Tancrede, 1992). Stomach and duodenum contains a low number of microorganisms, which is less than 103 cfu/g of luminal content (Guarner, 2006; O'Hara & Shanahan, 2006). The dominant microbiotas in this region of the GI tract include microbial populations of streptococci, lactobacilli, and yeasts (Ramakrishna, 2007). Stomach and duodenum have the acidic conditions making it uninhabitable for the majority of microorganisms (Guarner, 2006; Ramakrishna, 2007). Further, adhering to the lumen surface is difficult for the microorganisms owing to phasic propulsive mechanism in this area (Guarner, 2006). However, in comparison to the stomach and duodenum, jejunum and ileum contain a higher quantity of microbes. There is a progressive increase in the microbiota colonization from 10^4 in the jejunum to 10^7 cfu/g of luminal content in the ileum. The high quantity of microbiota colonization in the colon is due to the slow transition of contents, thus allowing the microbiota to proliferate and survive by fermenting indigestible material as well as endogenous secretions (O'Grady & Gibson, 2005; Guarner, 2006). The microbiota in this region of the GI tract consists of Gram-negative facultative aerobes, such as *Enterobacteriaceae* with a few obligate anaerobes, including *Bacteroides* and *Fusobacterium*. According to Guarner et al., the immune functioning can be influenced by the interactions of the gut microbiota with the lymphoid structures of the small intestinal mucosa (Guarner, 2006). The biggest population of microbiota in the GI tract is found in large intestine at a concentration of 10^{12} cfu/g of luminal content. The majority of the microbiota populations are strict anaerobes, such as *Bacteroides*, *Eubacterium*, *Bifidobacterium*, and *Peptostreptococcus*, with subdominant populations of facultative aerobes, including *Enterobacteriaceae*, streptococci, and lactobacilli. The microbiota is influenced by environmental conditions, diet, antibiotic use, and health status, among other factors. The categorization of the colonization pattern by microbiota of the GI tract has been used to describe the nature of the microorganisms. Autochthonous microorganisms originally colonize in the GI tract and will remain permanent residents of the GI tract, whereas allochthonous microorganisms inhabit the regions other than the area they originally colonize (O'Grady & Gibson, 2005). However, for both types, these microorganisms will inhabit areas of the GI tract based on suitable conditions for their survival. A third category, termed "opportunist," describes microorganisms that take advantage of conditions that suit their requirements.

2.2 Characteristics of the GI tract for probiotic delivery

It is essential to consider the complex physiology of the GI tract while designing delivery systems for probiotic encapsulation with the aim of providing controlled GI delivery. Three major profiles in the GI tract may influence the delivery of probiotic bacteria, including the pH profile, the oxygen profile, the type, and concentration of the enteric bacteria profile

in the GI tract. The presence of oxygen and the neutral pH in the oral cavity promote the high growth of aerophilic bacterial population. However, in the stomach, where the pH is around 2.0–3.0, low bacterial growth is supported. In the small intestinal areas, much less oxygen is available for aerophilic bacterial growth and thus the composition of the bacteria begins to change from aerophilic to anaerobic types with moderate bacterial growth. In the proximal colon the pH (5.0–6.0) provides conditions and nutrients for high anaerobic-type bacterial growth. However, in the distal colon, lower bacterial growth is seen due to various factors, including the neutral pH, the anaerobic conditions, nutrient scarcity, and the accumulation of wastes. Adhesion and growth of probiotic bacteria within the GI tract are also influenced by other factors such as antimicrobial agents secreted in the GI tract, mucoadhesion, interactions with the immune system (e.g., phagocytosis), and nutrient availability. There are various possible methods for the delivery of probiotics along the length of the GI tract, based on pH, time, peristaltic pressures, and fermentation (Cook, Tzortzis, Charalampopoulos, & Khutoryankiy, 2012). Bacteria are generally too large to diffuse from the traditional delivery devices; therefore the release of the bacteria from the capsules will be triggered by degradation, disintegration, or dissolution of the capsule. After ingestion of the encapsulated probiotic bacteria, greater viability losses are encountered if the bacteria are released into the acidic contents of the stomach. Further, more possibility of losing viable probiotic cells are seen due to gastric emptying time. These two factors depend upon age, buffers in the food and time since eating (wide range of pH in the stomach from eating to starving). In the intestine the pH regime is more toward alkalinity, and there is varying pH in the small intestine, the large intestine, and in colon. Hence, appropriate release mechanisms need to be designed. The large intestine consists of large number of indigenous bacterial species providing opportunity for targeted delivery, as some polysaccharides, such as pectin, are biodegraded by the colonic microflora enabling the possibility of using prebiotics for encapsulating probiotic bacteria and releasing them in the colon area (Chourasia & Jain, 2003). Furthermore, mucoadhesion enables the controlled release of encapsulated probiotic bacteria. It leads to increased residence time, improved contact with various biological membranes within the GI tract, and release at a specific site within the GI tract. In the case of mucoadhesive biopolymer, under physiological conditions, the adhesion results in the formation of aggregates due to electrostatic interactions and hydrogen bonding between the positively charged biopolymer molecules and the negatively charged intestinal mucosal layers (Dodou, Breedveld, & Wiering, 2005).

3 Encapsulation technologies for probiotics

There are many challenges, particularly with respect to the stability of probiotic bacterial cells during processing and storage. Encapsulation separates bacterial cells from their environment until they are released and thus improves their stability, extending the shelf-life, and providing sustained and controlled release. The architecture of encapsulation is generally divided into several arbitrary classifications. Matrix encapsulation or single-particle structure is the simplest structure, in which a sphere is surrounded by a wall membrane resembling that of a hen's egg where the core material is buried at varying depths inside the shell. Similarly, a number of core particles embedded in a continuous matrix of wall material with the several distinct cores within the same microcapsule known as aggregate structure can also be designed. Most of the literature on probiotic encapsulation was based on small-scale laboratory procedures with techniques that are gentle and nonaggressive toward the cells. In order to improve the shelf-life of probiotics, the first encapsulation techniques developed were to transform bacterial cultures into concentrated dry powder. Various encapsulation techniques such as spray-drying, freeze-drying and fluidized bed drying have been used in protecting the cell cultures. With specific reference to spray-drying, recent publications make reference to its effectiveness in protecting probiotic cells (Kitamura, Itoh, Echizen, & Satake, 2009; Riveros, Ferrer, & Borquez, 2009). This technique commonly used in food industry involves atomization of an aqueous or oily suspension of probiotics and carrier material into a drying gas, resulting in rapid evaporation of water (Rokka & Rantamaki, 2010). Water evaporation is defined as the difference between air inlet temperature and air outlet temperature. The spray-drying process is controlled not only by these temperatures, but also by the product feed and the gas flow. Despite the advantages of spray-drying technique, the high temperatures needed to facilitate water evaporation reduce the probiotics viability and their activity in the final product. The minimum air inlet temperature reported in the literature for probiotic encapsulation is 100°C, while the maximum is 170°C. The air at outlet temperature varies between 45°C and 105°C. At these temperatures, it is unlikely that the cells retain all their probiotic activity. Probiotic activity must be differentiated from probiotic survival. Probiotic activity takes into account the ability of cells to resist to GI environment and to adhere to intestinal mucosa, so it is important that the encapsulation technique does not reduce cell survival and does not inhibit their subsequent activities (Del Piano et al., 2008). To further improve the protection of probiotics against adverse conditions, other techniques have been introduced that provide probiotics with a physical barrier. The main aims of these techniques were to form gel beads or capsules by means of extrusion or emulsification techniques using hydrocolloids. Extrusion is a simple implementation, retaining a high number of cells. In extrusion technique an aqueous dispersion of

biomaterials (hydrocolloid) is mixed with probiotics. The mixture formed is fed into an extruder, usually a syringe. When the pressure is exerted on the plunger of the syringe, the contents of the syringe drops into a crosslinking solution. The diameter of the needle, and the distance between the crosslinking solution and the needle, determines the size and shape of the drops formed. Automated processes utilizing this principle are also available. The use of emulsification technique for probiotics encapsulation is also well known. In the emulsification technique, the mixture represents the discontinuous phase which is dispersed in a large volume of vegetable oil (continuous phase). The water-in-oil emulsion being formed is continuously homogenized by stirring. Size and the shape of the droplets formed are determined by the stirring speed (Gouin, 2004). The droplets are collected by settling, after the emulsion has been broken. Emulsification generates capsules having oily or aqueous droplets with a liquid core, while as extrusion it gives droplets in gel form named beads having a porous core network. As compared to beads, the capsules have sizes at least 100 times lower (Krasaekoopt et al., 2003a). However, emulsification is more expensive requiring additional raw materials such as vegetable oil and emulsifiers to stabilize the emulsion. Besides, difficulties in implementation of this process is seen, including emulsion instability, need for vigorous stirring which can be detrimental to cells survival, random incorporation of cells into the capsules, and inability to sterilize vegetable oil are seen. To improve beads or capsules stability, cross-linking with organic solvents (Cui, Goh, Kim, Choi, & Lee, 2001) coating with others biomaterials or adding additives or cryoprotectants in the mixture are made (Carvhalo et al., 2002).

3.1 Selecting the biomaterials for microencapsulation

Biomaterial has been described as "any natural material or not, having a direct contact with a living structure, intended to act with biological systems." These are the organic or inorganic macromolecules, with repeated chain of monomers linked by covalent bonds. After probiotic encapsulation the biomaterial used is intended to be in contact with the digestive tract of the host. Therefore general criteria for choosing biomaterials can be applied. The various issues involved when selecting biomaterials for probiotics encapsulation are (1) physicochemical properties, including chemical composition, mechanical strength, stability in gastric and intestinal fluids; (2) their toxicology; and (3) sterilization processes. There are various biomaterials for probiotics encapsulation available in the literature consisting of natural as well as synthetic polymers. Alginate is the most common biomaterial used for probiotics encapsulation (Anal & Singh, 2007). Other biomaterials include carrageenan, gelatin, starches, chitosan, locust bean gum, whey proteins, and cellulose acetate phthalate (CAP). Alginate is a naturally derived polysaccharide obtained from various species of algae. It is composed of two monosaccharide units: acid α-L-guluronic (G) and acid β-D-mannuronic (M) linked by β (1–4) glycosidic bonds (Dong, Wang, & Du, 2006). Functionality of alginate is determined by ratio of M/G monomers with the maximum gel strength of alginate having high proportion of block G (Hansen, Allan-Wojtas, Jin, & Paulson, 2002). Gels of alginate are insoluble in acidic media thus protecting the probiotic cells against acidity (Harnsilawat, Pongsawatmanit, & McClements, 2006). Carrageenan is a linear polymer of D-galactose units linked alternatively by α (1–3) and β (1–4) bonds. The safety of carrageenan has been approved by several government agencies, including the joint FAO/WHO food additives, FDA, and codex alimentarius. The various types of carrageenan known consist of kappa (κ), iota (ι), and lambda (λ) (Gaaloul, Turgeon, & Corredig, 2009). Between the carbons 3 and 6 of the D-galactose, κ-carrageenan and ι-carrageenan have an oxygen bridge responsible for the gelation and conformational transitions, while the λ-carrageenan does not have this bridge and thus is unable to form gel. A rise in temperature (60°C–80°C) is required to induce gelation in carrageenan (Mangione, Giacomazza, Bulone, Martorana, & San-Biagio, 2003). Due to its capacity to form gel that can entrap the cells, carrageenan are readily used to encapsulate probiotics. However, the gel hardens at room temperature and thus requires that the cell slurry should be added to the suspension between 40°C and 45°C. Whey proteins are used because of their amphoteric character. Adjusting the pH below their isoelectric point leads to the positive net charge of the proteins, causing an interaction with the negatively charged polysaccharides (Harnsilawat et al., 2006) and thus they can be easily mixed with negatively charged polysaccharides like alginate, carrageenan, or pectin. Gelatin is a protein obtained by partial hydrolysis of collagen. Gelatin has versatile functional properties, including formation of high viscosity solution in water, which on cooling sets to a gel (Rokka & Rantamaki, 2010).

Chitosan is a positively charged linear polysaccharide formed by deacetylation of chitin. It is composed of glucosamine units that can polymerize in the presence of anions and polyanions by means of a cross-link formation. Solubility of chitosan is pH dependent. Besides, it is water insoluble at a pH higher than 5.4, thus preventing the complete release of this biomaterial into the gut where pH is greater than 5.4. However, many reports have suggested that chitosan can form a membrane around a negatively charged polymer. Also, it is seen that the chitosan is effective as a coating agent of alginate gel beads, providing protection in simulated GI conditions, and thus acts as a good way to deliver viable bacterial cells to the colon (Krasaekoopt, Bhandari, & Deeth, 2004). Other biopolymers used to coat gel beads or to improve their stability

consists of whey proteins and gelatin (Anal & Singh, 2007). CAP is used for controlling drug release in the intestine due to its safe nature. Besides, it provides good protection for microorganisms in simulated GI conditions. It is insoluble at a pH below 5, but when the pH is greater than 6 it gets solubilized. This property is essential for probiotics encapsulation because the biomaterial must not dissolve into the stomach, but only into the gut. However, only capsules have been developed by emulsification using this biomaterial as it cannot form gel beads by ionotropic gelation.

Biomaterials used for encapsulation of probiotics must be stable in acidic environment and unstable in environment having a pH above 6, which is the minimum pH found in the intestinal lumen (beginning of the duodenum) (Lee & Heo, 2000). Hence, rigorous methodological approach is required while selecting the appropriate biomaterial such as proteins and polysaccharides. For instance, electrophoresis (SDS-PAGE) is used to assess the stability of proteins under varying conditions of pH. Similarly, FTIR-ATR can be used to study the stability of polysaccharides and other biomaterials by determining any change in its initial structure. Meanwhile, various reports have elucidated the interactions between the biomaterial mixtures (proteins-polysaccharides or polysaccharide-polysaccharide) and thus have shown that these biomaterial mixture can effectively encapsulate probiotics (Kailasapathy, 2009; Favaro-Trindade, Heinemann, & Pedroso, 2011). Searching for new encapsulation materials having the resistance in an acidic medium, and its disintegration or dissolution in environment with a pH above 6 will be of paramount importance in the near future. Further, these materials must meet the requirements of nontoxicity and compatibility with respect to probiotic cells.

4 Selecting the in vitro conditions for cells release

While encapsulating the probiotics, it is essential to ensure that the encapsulated biomaterials of probiotics are reliable in media simulating the gastric fluid, and then ensure that the encapsulated probiotics are released in media simulating the intestinal fluid. Experimental models, including "conventional model" and "dynamic model," have been described in the literature. These models simulate the GIT, evaluating the probiotic tolerance to acidic media, bile, and enzymes. The conventional model consists of a single reactor (or glass container) with the simulated gastric fluid or the simulated intestinal fluid that simulates either the stomach or the gut. The dynamic model is semiautomated. It consists of a series of reactors for stomach and gut, with respective volume. In dynamic model the temperature is maintained at 37°C and the pH is automatically controlled, thus maintaining the values of gastric and intestinal pH. Reactors are continuously stirred, with the addition of sterile culture medium to gastric reactor by a peristaltic pump that sequentially supplies it to the gut reactor. To obtain the mean transit time throughout the model, flow rate was set (Barmpalia-Davis, Geornaras, Kendall, & Sofos, 2008). More than half of the authors have suggested a preference for the NaCl medium. It maintains the viability of the microbial cells by providing an isotonic medium. Saline solution at a concentration of 9 g/L is reported by the American Society for Microbiology (ASM) for microbiological procedures such as microbial cells suspension or dilution (Chapin & Lauderdale, 2003). The ASM also recommends the addition of phosphate to NaCl medium. This provides a stable pH because of its buffering capacity, thus maintaining cell viability. However, the concentration of NaCl should be reduced (8–8.5 g/L). Regarding the pH of the gastric fluid, the values vary between 1 and 3, thus covering the values observed in human's stomach. For gastric enzyme, pepsin is sometimes used as a model. Pepsin is secreted in an inactive form (pepsinogen) that gets activated to pepsin in the presence of acidic medium requiring a pH under 5.6 (Tobey et al., 2001). Finally, regarding the exposure time, several values were observed, ranging from 20 to 240 min. Clinical studies conducted by Malmud, Fisher, Knight, and Rock (1982) have shown that a period of 120 min is sufficient to ensure the gastric emptying of 60% of a semisolid meal and 90% of a liquid meal (Singh et al., 2006). For the stay of probiotics in an artificial gastric medium, an exposure time of 120 min is reasonable. After that tests are conducted in the gut where bile and pancreatic enzymes are present and sodium salts are exclusively used as intestinal fluid at various concentrations. The phosphate-buffered saline can be a medium in which the salt concentrations have been adjusted or supplemented by other salts as needed. In reality, it consists mainly of NaCl in which other salts are added: NaCl (8.5 g/L), K_2HPO_4 (1.1 g/L), and KH_2PO_4 (0.32 g/L). Sometimes it consists of NaCl (8 g/L), Na_2HPO_4 (1.44 g/L), and KH_2PO_4 (0.24 g/L) (Chapin & Lauderdale, 2003). The pH values used are between 6.5 and 8 with the values reflecting the pH usually met in the gut (Shima, Morita, Yamashita, & Adachi, 2006). Regarding the concentrations of bile and enzymes, no published data allow specifying the exact levels, which may explain the variations observed from one author to another. The lack of published data on the transit time of the gut may explain the difference observed in the exposure time. Studies with radio-labeled food must be conducted to determine this transit time. The studies clearly show a lack of standard protocol in establishing the in vitro conditions for simulating the stomach or the gut. Searching a consensus in the standardization of protocols must be in compliance with the conditions prevailing into the GIT. Type of medium and its composition, choice of pH values, exposure time, the presence of gastric or intestinal enzymes, and the presence of bile are the essential factors to be taken into account. These factors should reflect reality as much as possible in humans.

5 Survival of entrapped LCS in simulated gastrointestinal conditions

The determination of probiotic cells viability under gastric and intestinal juices is vital as the feasibility and sustainability of the cells upon consumption is essential to confer desired health benefits to the host. Ayama et al. (2014) investigated the viability of lactic acid bacteria (LAB) with probiotic properties in simulated GIT condition using CM53 with 1%–3% alginate and 2% Hi-maize resistant starch. It was seen that encapsulation improved the survival of *Lactobacillus plantarum* CM53 in simulated GIT significantly ($P < .05$). Higher number of *L. plantarum* CM53 surviving in the 2% and 3% alginate bead were obtained for cells entrapped in 1% alginate bead than free cell after exposure to stomach gastric juice. When the beads were exposed to simulated small intestine, the cell survival after 4 h was 91.18%, 89.09%, 74.30%, and 69.09% of the initial population found in alginate beads produced by 3%, 2%, and 1% alginate and free cells, respectively. According to Annan, Borza, and Hansen (2008), control release of the microencapsulated cell load under the natural conditions found in the small intestine was due to pH-dependent behavior of the biopolymer used. The ability to release *L. plantarum* CM53 from PBS (pH 7.2) from alginate beads at the beginning of incubation (0 h) was 48.15 ± 0.06%, 45.70 ± 0.85%, and 45.25 ± 1.90% for 1%, 2%, and 3% alginate beads, respectively. Similarly, in the study of Guérin, Vuillemard, and Subirade (2003), an initial immobilized *Bifidobacterium bifidum* population of 10 log cfu/g in mixed alginate, pectin could reach the small intestine in numbers of 7.5 log cfu/g and providing the host with a beneficial health effect. Free and encapsulated *Bifidobacterium longum* cells were immersed in simulated gastric and intestinal fluids and studied over time for cell viability by Ji et al. (2019). It was seen that encapsulation enhanced the protection for *B. longum* incubated in simulated stomach and intestinal fluids. The viability of free *B. longum* significantly decreased by 3.75 log cfu at 60 min and could not be detected at 120 min, when the cells were exposed to pH 2.5, while as in alginate microcapsules decrease in cell viability by 3.88 log cfu at 30 min could not be detected at 60 min. In chitosan-coated alginate, microcapsules decrease in cell viability by 2.68 log cfu at 120 min when exposed to intestinal fluid with bile salt (1%) was also seen. The viability of free *B. longum* and alginate microcapsules was significantly decreased and could not be detected after the continuous simulated GI fluid treatment, while as chitosan-coated alginate microcapsules only showed decrease in viability by 2.76 and 3.91 log cfu at 180 and 240 min, respectively, showing that chitosan-coated microcapsules protected *B. longum* from gastric acid and bile salt injury. Gul, Osman, Atalar, and Ilyas (2019) studied the viability of *Lactobacillus casei Shirota* after freeze-drying. It was seen that free cells were completely inactivated after exposure to simulated GI conditions, while as the survival rates of microencapsulated *L. casei Shirota* were found high for all microcapsules. After 90 min of gastric treatment, the highest survivability of encapsulated LCS (*Lactobacillus casei Shirota*) was provided by reconstitute skim milk (RSM): gum arabic (GA) formulation with 0.49 log unit reduction in cell viability was seen. Fritzen-Freire, Prudêncio, and Pinto (2013) claimed that the symbiotic effect of wall materials beneficially effects the survival of cells and improved to resistance against GI conditions. It was seen that the addition of GA to RSM and maltodextrin efficiently increased the survivability of encapsulated *L. casei Shirota* during gastric conditions ($P < .05$). Fritzen-Freire, Prudêncio, and Pinto (2013) stated that the microcapsules produced from *Bifidobacterium BB-12* with RSM and inulin showed higher ($P < .05$) count when incubated in pH 2.0 after 3 h of exposure. Similarly, it was seen that whey protein isolate encapsulated microcapsules using freeze-drying can provide enough tolerance to probiotic cells in intestinal conditions indicating that encapsulation with the various carrier materials exert a protective effect on cells of probiotics against bile solution (Moayyedi, Eskandari, & Rad, 2018). (Zeashan, Afzaal, Saeed, & Anjum, 2020) compared the viability and stability of free and encapsulated probiotics under simulated technological and human GI conditions by encapsulating *L. acidophilus* using sodium alginate (SA), soy protein isolate (SPI), and SA-SPI. Free and encapsulated probiotics were subjected to simulated GI conditions. The survival of probiotics under simulated GI conditions was increased significantly ($P < .05$). It was seen that the buffering effect of microbeads prolonged their survival and stability under simulated conditions as the number of surviving probiotic cells encapsulated with SA, SPI, and SA-SPI over 120 days of product storage was 7.85 ± 0.39, 7.45 ± 0.37, and 8.50 ± 0.43 cfu/mL, respectively. In the case of free cells, the surviving cells were just 3.5 ± 0.18 cfu/mL over the storage period. The results showed a sudden drop in probiotics that were without encapsulation in contrast to the encapsulated cells at pH 7.5. Yasmin, Saeed, Pasha, and Zia (2019) also found that in simulated gastric conditions, probiotic survive better when encapsulated with different materials.

6 Conclusion

The growing appreciation of the importance of the gut microbiota on human health and wellness has led to a surge of interest in the development of oral delivery systems to encapsulate, protect, and deliver probiotics to the colon. In this chapter, we have highlighted some of the encapsulation technologies that have been used to enhance the stability of probiotics during exposure to various hostile conditions. Well-designed edible delivery systems could increase the efficiency of probiotic

to create a healthier gut microbiome resulting in improved human health and well-being. However, further studies are needed to ensure how they can be produced on a large scale. Moreover, they should be effective in protecting the probiotics during actual food production and storage. Further, to show the efficacy of delivery systems to protect and deliver probiotics to the actual human colon, in vivo human feeding studies are needed.

References

Anal, A. K., & Singh, H. (2007). Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. *Trends in Food Science and Technology*, *18*, 240–251.

Annan, N. T., Borza, A. D., & Hansen, L. T. (2008). Encapsulation in alginate-coated gelatin microspheres improves survival of the probiotic *Bifidobacterium adolescentis* 15703T during exposure to simulated gastrointestinal condition. *Food Research International*, *41*, 184–193.

Ayama, H., Sumpavapol, P., & Chanthachum, S. (2014). Effect of encapsulation of selected probiotic cell on survival in simulated gastrointestinal tract condition. *Songklanakarin Journal of Science and Technology*, *36*(3), 291–299.

Barmpalia-Davis, I. M., Geornaras, I., Kendall, P. A., & Sofos, J. N. (2008). Differences in survival among 13 *Listeria monocytogenes* strains in a dynamic model of the stomach and small intestine. *Applied and Environmental Microbiology*, *74*, 5563–5567.

Carvhalo, A. S., Silva, J., Ho, P., Teixeira, P., Malcata, F. X., & Gibbs, P. (2002). Survival of freeze-dried *Lactobacillus plantarum* and *Lactobacillus rhamnosus* during storage in the presence of protectants. *Biotechnology Letters*, *24*, 1587–1591.

Chapin, K. C., & Lauderdale, T. L. (2003). Reagents, stains and media: Bacteriology. In *Manual of clinical microbiology* (8th ed., pp. 15–35). Washington, DC, USA: American Society for Microbiology.

Chourasia, M. K., & Jain, S. K. (2003). Pharmaceutical approaches to colon targeted drug delivery systems. *Journal of Pharmacological Science*, *6*, 33–66.

Cook, M. T., Tzortzis, G., Charalampopoulos, D., & Khutoryankiy, V. V. (2012). Microencapsulation of probiotics for gastrointestinal delivery. *Journal of Controlled Release*, *162*, 56–67.

Cui, J. H., Goh, J. S., Kim, P. H., Choi, S. H., & Lee, B. J. (2001). Survival and stability of bifidobacteria loaded in alginate poly-L-lysine microparticle. *International Journal of Pharmaceutics*, *210*, 51–59.

Dave, R. A., & Shah, N. P. (1997). Effect of cysteine on the viability of yoghurt and probiotic bacteria in yoghurts made with commercial starter cultures. *International Dairy Journal*, *7*, 537–545.

Del Piano, M., Strozzi, P., Barba, M., Allesina, S., Deidda, F., Lorenzini, P., et al. (2008). *In vitro* sensitivity of probiotics to human pancreatic juice. *Journal of Clinical Gastroenterology*, *42*, S170–S173.

Dodou, D., Breedveld, P., & Wiering, P. A. (2005). Mucoadhesives in the gastrointestinal tract: Revisiting the literature for novel applications. *European Journal of Pharmaceutics and Biopharmaceutics*, *60*(1), 1–16.

Dong, Z., Wang, Q., & Du, Y. (2006). Alginate/gelatin blend films and their properties for drug controlled release. *Journal of Membrane Science*, *280*, 37–44.

Favaro-Trindade, C. S., Heinemann, R. J. B., & Pedroso, D. L. (2011). Developments in probiotic encapsulation. *CAB Reviews*, *6*, 1–8.

Fritzen-Freire, C. B., Prudêncio, E. S., Pinto, S. S., Muñoz, I. B., & Amboni, R. D. M. C. (2013). Effect of microencapsulation on survival of *Bifidobacterium* BB-12 exposed to simulated gastrointestinal conditions and heat treatments. *LWT - Food Science and Technology*, *50*(1), 39–44.

Fuller, R. (1989). Probiotics in man and animals. *Journal of Applied Bacteriology*, *66*, 365–378.

Gaaloul, S., Turgeon, S. L., & Corredig, M. (2009). Influence of shearing on the physical characteristics and rheological behaviour of an aqueous whey protein isolate-kappa-carrageenan mixture. *Food Hydrocolloids*, *23*, 1243–1252.

Gill, H. S., Cross, M. L., Rutherfurd, K. J., & Gopal, P. K. (2001). Dietary probiotic supplementation to enhance cellular immunity in the elderly. *British Journal of Biomedical Science*, *58*, 94–96.

Gouin, S. (2004). Microencapsulation: Industrial appraisal of existing technologies and trends. *Trends in Food Science and Technology*, *15*, 330–347.

Guarner, A. F. (2006). Enteric flora in health and diseases. *Digestion*, *73*(Suppl.), 5–12.

Guérin, D., Vuillemard, J. C., & Subirade, M. (2003). Protection of bifidobacteria encapsulated in polysaccharide protein gel beads against gastric juice and bile. *Journal of Food Protection*, *66*, 2076–2084.

Gul, O., & Atalar, I. (2019). Effect of different encapsulating agent combinations on viability of *Lactobacillus casei Shirota* during storage, in simulated gastrointestinal conditions and dairy dessert. *Food Science and Technology International*, *25*(7), 608–617. doi: 10.1177/1082013219853462.

Hansen, L. T., Allan-Wojtas, P. M., Jin, Y. L., & Paulson, A. T. (2002). Survival of Ca^{2+}-alginate microencapsulated *Bifidobacterium* spp. in milk and simulated gastrointestinal conditions. *Food microbiology*, *19*, 35–45.

Harnsilawat, T., Pongsawatmanit, R., & McClements, D. J. (2006). Characterization of β-lactoglobulin-sodium alginate interactions in aqueous solutions: A calorimetry, light scattering, electrophoretic mobility and solubility study. *Food Hydrocolloids*, *20*, 577–585.

Hughes, D. B., & Hoover, D. G. (1991). Bifidobacteria: Their potential for use in American dairy products. *Food Technology*, *45*, 74–83.

Isolauri, E., Arvola, T., Sutas, Y., Moilanen, E., & Salminen, S. (2000a). Probiotics in the management of a topic eczema. *Clinical and Experimental Allergy*, *30*, 1604–1610.

Isolauri, E., Arvola, T., Sutas, Y., Moilanen, E., & Salminen, S. (2000b). Probiotics in the management of atopic eczema. *Clinical and Experimental Allergy*, *30*, 1604–1610.

Ji, R., Wu, J., Zhang, J., Wang, T., Zhang, X., & Wang, J. (2019). Extending viability of *Bifidobacterium longum* in chitosan-coated alginate microcapsules using emulsification and internal gelation encapsulation technology. *Frontiers in Microbiology*, *10*, 1389. doi: 10.3389/fmicb.2019.01389.

Kailasapathy, K. (2009). Encapsulation technologies for functional foods and nutraceutical product development. *CAB Reviews*, *6*, 1–19.

Kailasapathy, K., & Chin, J. C. (2000). Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. *Immunology and Cell Biology*, *78*, 80–88.

Kailasapathy, K., & Sultana, K. (2003). Survival and β-D-galactosidase activity of encapsulated and free *Lactobacillus acidophilus* and *Bifidobacterium lactis* in ice cream. *Australian Journal of Dairy Technology*, *58*, 223–227.

Kailasapathy, K., & Supriadi, D. (1996). Effect of whey protein concentrate on the survival of *Lactobacillus acidophilus* in lactose hydrolyzed yoghurt during refrigerated storage. *Milchwissenschaft*, *51*, 565–569.

Kalliomaki, M., Salminen, S., Poussa, T., Arvilommi, H., & Isolauri, E. (2003). Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet*, *361*, 1869–1871.

Kitamura, Y., Itoh, H., Echizen, H., & Satake, T. (2009). Experimental vacuum spray drying of probiotic foods included with lactic acid bacteria. *Journal of Food Processing and Preservation*, *33*, 714–726.

Krasaekoopt, W., Bhandari, B., & Deeth, H. (2003a). Evaluation of encapsulation techniques of probiotics for yoghurt. *International Dairy Journal*, *13*, 3–13.

Krasaekoopt, W., Bhandari, B., & Deeth, H. (2003b). Evaluation of encapsulation techniques of probiotic for yoghurt. *International Dairy Journal*, *13*, 3–13.

Krasaekoopt, W., Bhandari, B., & Deeth, H. (2004). The influence of coating on some properties of alginate beads and survivability of microencapsulated probiotic bacteria. *International Dairy Journal*, *14*, 737–743.

Lee, K. Y., & Heo, T. R. (2000). Survival of *Bifidobacterium longum* in calcium alginate beads in simulated gastric juices and bile salt solution. *Applied and Environmental Microbiology*, *66*, 869–873.

Malmud, L. S., Fisher, R. S., Knight, L. C., & Rock, E. (1982). Scintigraphic evaluation of gastric emptying. *Seminars in Nuclear Medicine*, *12*, 116–125.

Mangione, M. R., Giacomazza, D., Bulone, D., Martorana, V., & San-Biagio, P. L. (2003). Thermoreversible gelation of k-Carrageenan: Relation between conformational transition and aggregation. *Biophysical Chemistry*, *104*, 95–105.

Moayyedi, M., Eskandari, M. H., Rad, A. H. E., et al. (2018). Effect of drying methods (electrospraying, freeze drying and spray drying on survival and viability of microencapsulated *Lactobacillus rhamnosus* ATCC 7469. *Journal of Functional Foods*, *40*, 391–399.

O'Grady, B., & Gibson, G. R. (2005). Microbiota of the human gut. In A. Tamime (Ed.), *Probiotic dairy products*. Oxford: Blackwell Publishers Ltd.

O'Hara, A. M., & Shanahan, F. (2006). The gut flora as a forgotten organ. *EMBO Reports*, *7*(7), 688–693.

Paturi, G. (2007). *Probiotic characteristics of Lactobacillus acidophilus and Lactobacillus paracasei and their effects on immune response and gene expression in mice*. NSW, Australia: University of Western Sydney. http://handle.uws.edu.au:8081/1959.7/17783 Ph.D. thesis.

Ramakrishna, B. S. (2007). The normal bacterial flora of the human intestine and its regulation. *Journal of Clinical Gastroenterology*, *41*(Suppl. 1), S1–S6.

Reid, G., Sanders, M. E., Gaskins, H. R., Gibson, G. R., Mercenier, A., Rastall, R., et al. (2003). New scientific paradigms for probiotics and prebiotics. *Journal of Clinical Gastroenterology*, *37*, 105–118.

Rinkinen, M., Jalara, K., Westermark, E., Salminen, S., & Ouwehand, A. C. (2003). Interaction between probiotic LAB and Canine enteric pathogens: A risk factor for intestinal *Enterococcus faecium* colonization. *Veterinary Microbiology*, *92*, 111–119.

Riveros, B., Ferrer, J., & Borquez, R. (2009). Spraydrying of a vaginal probiotic strain of *Lactobacillus acidophilus*. *Drying Technology*, *27*, 123–132.

Rokka, S., & Rantamaki, P. (2010). Protecting probiotic bacteria by microencapsulation: Challenges for industrial applications. *European Food Research and Technology*, *231*, 1–12.

Shima, M., Morita, Y., Yamashita, M., & Adachi, S. (2006). Protection of *Lactobacillus acidophilus* from the low pH of a model gastric juice by incorporation in a W/O/W emulsion. *Food Hydrocolloids*, *20*, 1164–1169.

Singh, S. J., Gibbons, N. J., Blackshaw, P. E., Vincent, M., Walker, J., & Perkins, A. C. (2006). Gastric emptying of solids in normal children. A preliminary report. *Journal of Pediatric Surgery*, *41*, 413–417.

Tancrede, C. (1992). Role of human microflora in health and diseases. *European Journal of Clinical and Infectious Diseases*, *11*, 1012–1015.

Tobey, N. A., Hosseini, S. S., Caymaz-Bor, C., Wyatt, H. R., Orlando, G. S., & Orlando, R. C. (2001). The role of pepsin in acid injury to esophageal epithelium. *The American Journal of Gastroenterology*, *96*, 3062–3070.

Yasmin, I., Saeed, M., Pasha, I., & Zia, M. A. (2019). Development of whey protein concentrate-pectin-alginate based delivery system to improve survival of *B. longum* BL-05 in simulated gastrointestinal conditions. *Probiotics and Antimicrobial Proteins*, *11*(2), 413–426.

Zeashan, M., Afzaal, M., Saeed, F., & Anjum, F. M. (2020). Survival and behavior of free and encapsulated probiotic bacteria under simulated human gastrointestinal and technological conditions. *Food Science and Nutrition*, *8*(5), 2419–2426. doi: 10.1002/fsn3.1531.

Chapter 4

Next-Generation Probiotics

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1 Introduction

The complex and dynamic microbial community residing within the gastrointestinal tract (GIT), referred to as gut microbiota, dominated by strictly anaerobic bacteria (Qin et al., 2010), which plays a vital role in maintaining intestinal homeostasis, regulating host immunity (Gensollen, Iyer, Kasper, & Blumberg, 2016), and metabolism (Aron-Wisnewsky et al., 2020). There are also archaea, eukarya, and viruses but their importance to human health has been less studied (Schroeder & Bäckhed, 2016). Moreover, the commensal bacteria have been reported to modulate intestinal integrity and protect against pathogens (Bäumler & Sperandio 2016). The major gut bacterial phyla in the healthy gut are Firmicutes and Bacteroidetes followed by Proteobacteria, Actinobacteria, and Verrucomicrobia, although their relative abundances are considerably diverse at their species level (Arumugam et al., 2011). The Firmicutes phylum consists primarily members of genera *Clostridium*, *Lactobacillus*, and *Ruminococcus*, as well as the butyrate producers *Eubacterium*, *Fecalibacterium*, and *Roseburia.* Bacteroidetes phylum consists of predominant genera of *Bacteroides*, *Prevotella*, and *Xylanibacter* that are recognized for their ability to degrade dietary fibers. The phylum Actinobacteria represents primarily the genus *Bifidobacterium*. The phylum Proteobacteria includes the genus *Escherichia* and *Desulfovibrio*, while the phylum Verrucomicrobia so far comprises only the mucus-degrading genus *Akkermansia* (Schroeder & Bäckhed, 2016). A thick layer of mucus separates the intestinal epithelium from resident microbes (Belkaid & Naik, 2013). The intestinal mucus comprises primarily of mucin that represents threonine, proline, and serine-rich peptide backbone agglomerates with glycans such as fucose, galactose, *N*-acetylgalactosamine (GalNAc), and *N*-acetylglucosamine (GlcNAc), although in some contexts altered with sialic acid (*N*-acetylneuraminic acid) and sulfate via *O*-glycosidic linkages and secreted by the goblet cells (Ottman et al., 2017). The intestinal mucus layer is essential for maintaining the commensal microbiota composition and regulating host immune system-microbe interactions. It may act as a source of carbohydrates to the gut microbiota. For instance, human symbionts, including *Bacteroides fragilis* and *Akkermansia*, colonize the mucus and utilize it as a carbon and energy source. They do not compete with other microbiota for nutrients derived from host food consumption and protect the host against intestinal pathogens by stopping bacterial attachment or invasion and inserting toxins to the mucous surface (Collado, Derrien, Isolauri, de Vos, & Salminen, 2007). Moreover, these symbionts along with their metabolites not only colonize the host and compete with transient pathogens via a competitive exclusion mechanism (Belzer & De Vos, 2012) but also have an impact on the host metabolic health through pathways that influence satiety, gut permeability, and immune function (Joyce & Gahan, 2014).

Multiple factors affect the composition of the intestinal microbiota, including host genetics, age, gender, diet, environmental factors, and therapies, especially antibiotics across the lifetime (Hasan & Yang, 2019). These factors contribute to the diversity and uniqueness of gut microbiota to each individual (Li et al., 2020) and may help one to determine the influence of gut microbiota on human health and disease. However, the abnormal change in the microbial community (dysbiosis) has been associated with several health problems, including inflammatory disorders such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), allergy, autoimmunity (Natividad & Verdu, 2013), obesity and diabetes(Wen & Duffy, 2017), and cardiovascular disease and hypertension (Hasan & Yang, 2019). For instance, the increased abundance of Proteobacteria contributed to increased gut permeability (leaky gut) and inflammation in the host (Tsai et al., 2019). Moreover, Crovesy, Masterson, & Rosado, 2020 reported the altered gut microbiota profiles with an increase in the Firmicutes/Bacteroidetes ratio in obese individuals. The increased abundance of Firmicutes, Fusobacteria, Proteobacteria, and

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00004-6
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some species of *Lactobacillus* was observed, while the decreased abundance of Bacteroidetes, *Akkermansia muciniphila*, *Faecalibacterium prausnitzii,* including some *Lactobacillus* species such as *Lactobacillus plantarum*, and *Lactobacillus paracasei* was observed in obese individuals as compared to normal-weight individuals (Crovesy, Masterson, & Rosado, 2020). The altered microbiota could be a clinical prognostic signature of dysbiosis and associated diseases.

A significant advance in the field of host-microbiome interaction has enabled us to understand the concepts of microbiota colonization and its mediated response to several diseases in the host. This observation has promoted the development of microbiome-targeted therapeutic strategies in dysbiosis-related diseases, including the administration of probiotics, prebiotics, and synbiotics or by repopulating with commensal bacterial species through an infusion of fecal microbiota transplantation (FMT) (Markowiak & Śliżewska, 2017). However, the prophylactic and therapeutic efficacy of traditional probiotics, prebiotics, and synbiotics on the alleviation of certain diseases are marginal and controversial and have not yet had a significant impact on the improvement of specific diseases (Tsai et al., 2019). Although FMT is effective in the treatment of recurrent *Clostridium difficile* infection, its use in other metabolic diseases remains elusive (Sbahi & Di Palma, 2016).

Therefore the use of next-generation probiotic (NGP) may provide a promising alternative to maintain gut homeostasis and target-specific health issues and needs. Meanwhile, NGP belongs primarily to commensal gut bacteria that can be developed as either a dietary supplement or novel drug that faces a new challenge to their efficacy, safety, technological robustness, and regulatory framework (Saarela, 2019). The emerging NGP candidate and their probable mechanism of probiotic activities in the amelioration of disease along with their safety, technological robustness, and regulatory challenges are discussed in this chapter.

2 Next-generation probiotics

Initially, the gut microbiota was characterized by conventional culture-based methods, now these have been replaced by cheap, rapid, efficient, and easily accessible high-throughput sequencing methods (Aron-Wisnewsky et al., 2020). The recent advancement in metagenomics, metatranscriptomics, metaproteomics, and metabolomics applications has broadened the understanding of the composition and impact of the human gut microbiota in health and diseases. This fosters the use of commensal bacteria as probiotic to maintain homeostasis within the GIT as a novel health-promoting approach (Zhang, Li, Cheng, Buch, & Zhang, 2019). This unlocks the way for a new class of probiotics, termed next-generation probiotics (NGPs), which can also be assigned as live biotherapeutics products (LBPs) (O'Toole, Marchesi, & Hill, 2017). NGP explicitly conforms to the FAO/WHO (Food and Agriculture Organization/World Health Organization) definition of the probiotic as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2002). NGP also fit with the US Food and Drug Administration (FDA) definition of the LBP: "a biological product that contains live organisms; is applicable to the prevention, treatment or cure of a disease or condition of human beings; and is not a vaccine" (O'Toole et al., 2017). Therefore NGP can allude as live biotherapeutic products (LBPs) (O'Toole et al., 2017). At this point the classification of certain microorganisms as NGPs differs from traditional probiotics on the delivery route to the market either as a novel drug or nutraceutical products (Fig. 4.1).

2.1 Need for next-generation probiotics

Traditional probiotic microorganisms include the scanty of genera mainly consisting of *Lactobacillus* and *Bifidobacterium* from Firmicutes and Actinobacteria phylum, respectively, including some genera of *Streptococcus*, *Bacillus* from Firmicutes phylum, *Escherichia coli* from Proteobacteria phylum, and *Saccharomyces cerevisiae* (yeast). They are generally isolated from the fecal microbiota or fermented foods (Suez, Zmora, Segal, & Elinav, 2019). *Lactobacillus* and *Bifidobacterium* are generally recognized as safe (GRAS) for their long history of safe use and are recognized as such for most of their strains by regulatory authorities.

Despite the popularity of these probiotics, there is no concrete evidence of how successful they are in improving human health, promoting longevity, or reducing infections (Pamer, 2016). The evidence of effective gut mucosal colonization by these probiotics, which relies mainly on stool microbiome assessments that only partially correlate their impacts on the host and microbiome, is refuted by culture-based techniques. The reason behind their ineffective colonization in many healthy human GI tracts may be their link with locally etched microbiome habitats, which differ significantly in physiological conditions of the human intestinal tract (Zmora et al., 2018). Moreover, the efficacy of traditional prophylaxis probiotics often points to contradictory conclusions due to their effect being marginal, ambiguous, and debatable (Koutnikova et al., 2019; Tsai et al., 2019). They are not found completely safe for all groups of people. Although their risk of infection and/or morbidity is rare, but they have been associated with the risk of infection (Quin et al., 2018), meningitis, and gastroschisis

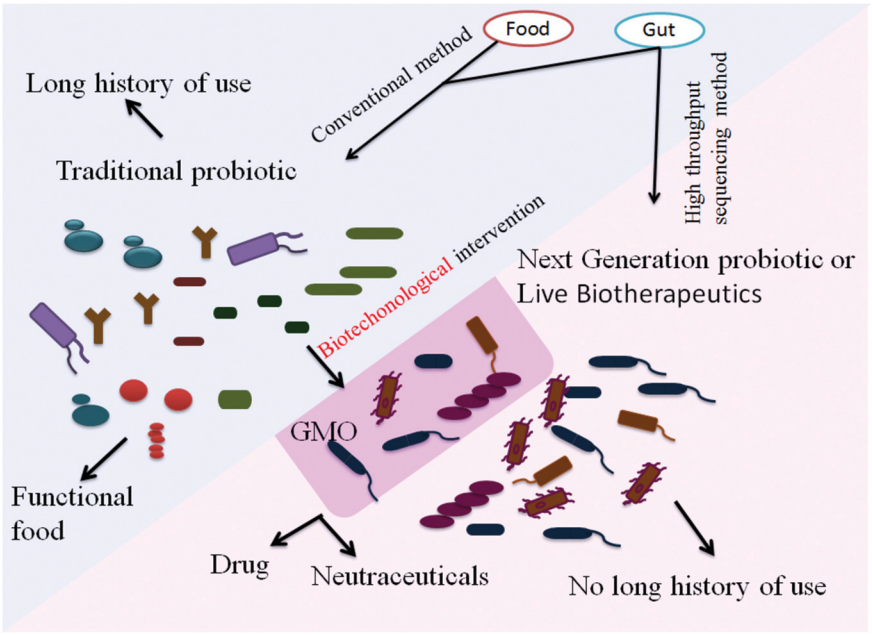


FIGURE 4.1 Differences in traditional probiotics and next-generation probiotics.

(Sanders et al., 2010) in infants and increase risk of systemic and localized infections, including sepsis, endocarditis, deleterious metabolic activities, and gastrointestinal side effects (Doron & Snydman, 2015), via bacteremia (bacteria in the blood) or fungemia (fungi in the blood) (Kothari, Patel, & Kim, 2019) in postoperative, hospitalized, or immunocompromised patients (Suez et al., 2019). The conflicting report also challenges probiotics' efficacy in antibiotic-associated diarrhea (AAD) where they reshape alter gut microbiota induced by antibiotic treatment in some but not all human studies (Suez, Zmora, & Elinav, 2020). Similarly, probiotics treatment has shown to modulate fecal microbiome composition in some reports, while others observed no effect (Zmora et al., 2018). Another limitation of traditional probiotic is the characterization used by the industry based on low virulence, viability, and lack of any unpleasant taste in food and universally provided as a "one-size-fits-all" intervention (Zmora, Suez, & Elinav, 2019).

Instead of administering probiotics universally as a "one-size-fits-all" supplement to cause health-promoting effects, they could be adapted to the patient's need for targeting specific diseases. Furthermore, clinical and statistical heterogeneity and unreliable estimates significantly decrease major clinical recommendations of traditional probiotics and demand concrete evidence of their claimed benefits. Overall, traditional probiotics exhibit limited impacts on the human intestinal microbiome and amelioration of diseases as do not aim against specific diseases (Petrof et al., 2012; Manzanares, Lemieux, Langlois, & Wischmeyer, 2016). Therefore commensal-based treatments could be considered as NGPs and may potentially outweigh the widely used strains of traditional probiotics in terms of certain health benefits and target-specific health issues and needs.

3 Candidates for next-generation probiotics

Numerous microbial species (candidates for the NGP) are gaining growing attention for their role in regulating the gut microbiota and treating various host diseases as well as several potential NGPs are under development. The candidates for NGP can be explored from the commensal bacteria that are linked to gut homeostasis and human health, including strains of *A. muciniphila*, *B. fragilis*, *F. prausnitzii*, and *Eubacterium hallii* and *Parabacteroides goldsteinii* and also genetically modified strains. These could be powerful and innovative alternative therapies for infection and metabolic disease management.

3.1 *Akkermansia muciniphila*

A. muciniphila is a key symbiont member of the intestinal microbiota and specialized in mucin degradation. It is characterized as beneficial microbes for the treatments of several metabolic disorders in the host and could be considered as a promising candidate for NGP.

3.1.1 Characteristics

A. muciniphila is a Gram-negative, strictly anaerobic, oval-shaped, nonendospore former, nonmotile, obligatory chemoorganotroph bacterium that belongs to the phylum Verrucomicrobia, class Verrucomicrobiae, order Verrucomicrobiales, and family Verrucomicrobiaceae. It can occur singly in chains or forms aggregates and its cellular size ranges from 0.6 to 1.0 μm, depending on the cultured medium used (Derrien, Vaughan, Plugge, & de Vos, 2004; Gómez-Gallego, Pohl, Salminen, De Vos, & Kneifel, 2016).

A. muciniphila colonizes the mucus layer of the intestinal epithelial cell and uses it as a sole source of carbon and nitrogen by producing mucin degrading enzymes (van Passel et al., 2011). Several mucin degrading enzymes, including glycosyl hydrolases, proteases, sulfatases, and sialidases, were upregulated during the growth of *A. muciniphila* in the presence of mucin as compared to growth on glucose. Similarly, the most gene involved in glycolysis, energy metabolic pathways, and protein secretion systems, including outer membrane protein, Amuc_1100, were upregulated under mucin-depleted conditions (Shin et al., 2019) and a similar result at the proteome level was also observed (Ottman et al., 2017). This bacterium is capable of converting *N*-acetylgalactosamine, *N*-acetylglucosamine, fucose, glucose, and other abundant sugar components in mucus into acetate, propionate, and to a lesser extent 1,2-propanediol and succinate and able to release sulfate in a free form (Derrien et al., 2004). Additionally, *A. muciniphila* ables to synthesize all essential amino acids, except L-threonine, which is among the most prevalent amino acids in the protein structure of the mucin (Ottman et al., 2017). It requires a complex animal-based medium containing digested animal proteins such as gastric mucin, brain–heart infusion, or Columbia media for the growth (Derrien et al., 2004). This complex mucin-based medium can be replaced by a human-compatible synthetic medium in which mucin is substituted by a combination of sugar such as glucose, *N*-acetylglucosamine, and a protein source such as soy peptone and threonine (Plovier et al., 2017). Additionally, the aerotolerant nature of *A. muciniphila* was described later rather than a strict anaerobe (Reunanen et al., 2015) that is capable of tolerating nanomolar levels of oxygen via cytochrome *bd* complex as a terminal oxidase. In the presence of oxygen, there is a shift in the metabolic process toward a higher acetate-to-propionate ratio, resulting in more ATP and NADH and ultimately resulting in a slightly higher growth rate and yield. This added energy is used by *A. muciniphila* to outcompete strict anaerobes in the mucus layer of its niche (Ouwerkerk et al., 2016). This bacterium can grow without vitamins within a temperature range of 20°C–40°C and pH ranges from 5.5 to 8, with an optimum temperature at 40°C and pH of 6.5 (Derrien et al., 2004).

A. muciniphila is abundant in the intestines of animal kingdom varying from humans to nonmammals (Belzer & De Vos, 2012). This species of bacteria is abundant in human intestinal tract and represents 0.01% to nearly 4% of the total bacterial load of the gut (van Passel et al., 2011). Besides the colon, *A. muciniphila* is found in human milk, oral cavity, pancreas, biliary system, small intestine, and appendix (Geerlings, Kostopoulos, De Vos, & Belzer, 2018). It is present in the human gut across different stages of life with colonization in early life and develops within a year to a level equal to that identified in healthy adults (Derrien, Collado, Ben-Amor, Salminen, & de Vos, 2008) but slightly declines in old people (Collado et al., 2007). Furthermore, *A. muciniphila* is capable of surviving an active acid resistance system and able to degrade human milk oligosaccharides. This helps *A. muciniphila* in initial colonization of the GIT and strengthening of the gut barrier that would likely to benefit the infant's gut health (Ottman, 2015; Zhang et al., 2019; Korpela et al., 2018).

3.1.2 Effects of Akkermansia muciniphila in host health

A. muciniphila is an important member of gut microbiota that has been inversely linked with various metabolic diseases such as IBD, obesity, type 2 diabetes, autism, atopy, and related diseases (Table 4.1).

Accumulating evidence indicates the beneficial role of *A. muciniphila* in prevention and alleviation of metabolic disorders (Xu et al., 2020). A metabolic disorder occurs when the normal metabolism processes of carbohydrates, amino acids, and fatty acids are disrupted and may involve a range of clinical symptoms, including dysglycemia, increased blood pressure, abnormal cholesterol or triglyceride levels, and obesity (mainly central adiposity) and eventually increases the risk of developing cardiovascular disease as well as diabetes (Alberti et al., 2009) and has also been related to cognitive impairment, including decreased mental capacity and memory deficits (Huynh, Schneider, & Gareau, 2016). *A. muciniphila* (active or pasteurized) could be an indicator of healthy metabolic status as it maintains intestinal barrier, reduces systemic inflammation, modulates glucose homeostasis (Dao et al., 2016; Zhang et al., 2018), ameliorates obesity-associated neurodevelopmental disorders (Yang et al., 2019), and regulates other metabolism homeostasis and gut microbial composition through different signaling routes (Xu et al., 2020). The decrease in *A. muciniphila* might be used as a potential marker for the early diagnosis of type 2 diabetes (Yassour et al., 2016; Xu et al., 2020) as it had a substantially lower abundance in both prediabetes or newly diagnosed type 2 diabetes groups (Zhang et al., 2013) except for one study showing an increase in *A. muciniphila* in type 2 diabetic subjects (Qin et al., 2012).

TABLE 4.1 Positive effects of *Akkermansia muciniphila* on health.

Target conditions	Study type	Treatment	Analysis type	Findings	References
Obesity	Diet-induced obese mice	Oral gavage of 2×10^8 CFU/180 µL of pasteurized *A. muciniphila* (ATTC BAA-835) for 5 weeks	–	Pasteurized *A. muciniphila* reduced body-weight and fat mass gain through reduction of carbohydrates absorption and enhanced intestinal epithelial turnover	Depommier et al., 2020
Obesity	Overweight and obese adults (N = 49, including 41 women)	Calorie restriction	Fecal microbiota analysis by quantitative metagenomics	*A. muciniphila* improved clinical parameters, including fasting glucose, waist-to-hip ratio, and subcutaneous adipocyte diameter	Dao et al. (2016)
Obesity	Overweight/obese insulin-resistant volunteers (n = 40)	Ingest either 10^{10} cells of live *A. muciniphila* or 10^{10} cells of pasteurized *A. muciniphila* for 3 months	Fecal microbial analysis by qPCR	*A. muciniphila* live or pasteurized reduced the levels of the relevant blood markers for liver dysfunction and inflammation	Depommier et al. (2019)
Obesity	High-fat diet-induced obese mice	Oral administration of 200 µL alive *A. muciniphila MucT* (ATCC BAA-835) and 200 µL extracellular vesicles for 5 weeks		*A. muciniphila* and its extracellular vesicles improved the intestinal barrier integrity, inflammation, energy balance, and blood parameters (i.e., lipid profile and glucose level)	Ashrafian et al. (2019)
Obesity	Normal-weight (n = 34) and overweight (n = 16) pregnant women		Fecal microbiota composition analysis by quantitative real-time PCR	*A. muciniphila* lowered in women with excessive weight gain than in women with normal weight gain during pregnancy	Santacruz et al. (2010)
Obesity	Overweight or obese children (n = 20) and normal-weight children (n = 20)		Fecal microbiota analysis by quantitative PCR (qPCR) and terminal restriction fragment length polymorphism	Lower abundance of *A. muciniphila* in the obese/overweight children compared with BMI within the normal-range children	Karlsson et al. (2012)
Obesity	Hippocampus-dependent contextual/spatial learning and memory impaired in HFD-fed mice	Oral administration of 200 µL suspension of *A. muciniphila* (ATCC BAA845) at 5×10^9 CFU/mL for 28 days	Fecal microbiota analysis by 16S rRNA gene sequencing	Lower abundance of *A. muciniphila* in HFD-fed mice. *A. muciniphila* ameliorated defects in learning and memory	Yang et al. (2019)
Obesity	High-fat diet-induced obesity mice		Gut microbiota analysis from cecal content by real-time quantitative PCR (qPCR)	*Lower abundance A. muciniphila* with HFD-feeding. *A. muciniphila* modulated lipid metabolism and inflammation markers	Schneeberger et al. (2015)
Obesity and type 2 diabetes	Genetic (ob/ob) and High-fat diet-fed obese and type 2 diabetic mice	Oral administration of *A. muciniphila* (2×10^8 CFU/0.2 mL) or heat-killed *A. muciniphila* for 4 weeks	Gut microbiota from cecal contents analysis using real-time quantitative PCR (qPCR) and the MITChip	A. *muciniphila* decreased in obese and type 2 diabetic mice. A. *muciniphila* reversed high-fat diet-induced metabolic disorders and insulin resistance	Everard et al. (2013)

(*Continued*)

TABLE 4.1 Positive effects of *Akkermansia muciniphila* on health. (*Cont.*)

Target conditions	Study type	Treatment	Analysis type	Findings	References
Obesity and type 2 diabetes	Obese and diabetic mice	Pasteurized *A. muciniphila* and outer membrane of A. *muciniphila* derived protein Amuc_1100		Pasteurized A. *muciniphila* alleviated fat mass development, insulin resistance, and dyslipidemia. Amuc_1100 improved gut barrier	Plovier et al. (2017)
Diabetes	High fat–diet (HFD) mice	Metformin for 6 weeks	16S rRNA gene sequences with 454 pyrosequencing	Metformin improves the abundance of *Akkermansia* that improved glucose homeostasis and attenuated adipose tissue inflammation	Shin et al. (2014)
Gut permeability	HFD-fed mice	Oral administration of extracellular vesicles of *A. muciniphila* ATCC BAA-835 (AmEVs) for 2 weeks		Extracellular vesicles of *A. muciniphila* improved metabolic functions, reduced the gut permeability, and regulated intestinal barrier integrity	Chelakkot et al. (2018)
Inflammation	*In vitro* models			*A. muciniphila* adhered to the intestinal epithelium and strengthened enterocyte monolayer integrity by promoting colonic mucus turnover and lowering LPS uptake	Reunanen et al. (2015)
Inflammation	Normal-chow diet-fed mice	Oral gavage of *A. muciniphila* (ATCC BAA-835) at 2×10^8 colony-forming units (CFUs)/200 µL for 5 weeks		*A. muciniphila* decreased metabolic endotoxemia, as well as the inhibited proinflammatory pathways, ER stress and lipogenesis in insulin-responsive tissues, leading to improved insulin action and glucose tolerance	Zhao et al. (2017)
Inflammation	Germ-free *Il10*$^{-/-}$ mice	Colonization with *A. muciniphila* strain ATCC BAA-835	Microbiota analysis by FISH using fluorescently labeled oligonucleotide probes	*A. muciniphila* showed no signs of intestinal inflammation based on body weight change, histopathological scoring, and inflammatory markers	Ring et al. (2019)
Colitis-associated colorectal cancer (CAC)	Dextran sulfate sodium to induce colitis, followed by azoxymethane to establish colitis-associated colorectal cancer	Oral administration of pasteurized *A. muciniphila* (1.5×10^8 CFU) or the protein Amuc_1100 (3 µg)	Fecal microbiota analysis by 16S rRNA sequencing on Illumina MiSeq platform	*A. muciniphila* and Amuc_1100 improved colitis and blunted colitis-associated colorectal cancer	Wang et al., 2020
Ulcerative colitis (UC)	Patients either with UC in remission ($n = 6$) or with active disease ($n = 6$), and in healthy controls ($n = 6$)		Dice cluster analysis and principal component analysis of fecal microbiota profiles obtained by denaturing gradient gel electrophoresis and quantitative PCR, respectively	Alterations in the composition of the Gram-negative bacterial population, as well as reduced numbers of lactobacilli and *A. muciniphila* in Ulcerative colitis	Vigsnæs, Brynskov, Steenholdt, Wilcks, & Licht (2012)

Ulcerative colitis (UC)	Patients with active UC (n = 20), quiescent UC (n = 14), and healthy controls (n = 20)	–	Gut microbiota analysis by RT-PCR from colonic biopsies and mucus brushings samples	*A. muciniphila* confirmed an inverse relationship between its abundance in the mucus gel layer and active inflammation	Earley et al. (2019)
Colitis	DSS-induced colitis mice	Oral administration of *A. muciniphila* (5×10^8 CFU/mouse), and *A. muciniphila*-derived EV (AmEV, 100 mg/mouse)	Microbiota analysis by metagenome sequencing	*A. muciniphila*–derived extracellular vesicles negatively linked with colitis, protected DSS-induced colitis phenotypes, and also regulated intestinal immunity and homeostasis	Kang et al., 2013
Inflammatory bowel disease (IBD)	CD patients (n = 26), UC patients (n = 20), and control (n = 20)		Microbiota analysis by real-time PCR from biopsies (ileal, proximal colon, distal colon) sample	*A. muciniphila* reduced many fold in ulcerative colitis and Crohn's disease patients	Png et al. (2010)
Atherosclerosis	Apolipoprotein E–deficient ($Apoe^{-/-}$) mice	Oral gavage of 5×10^9 CFU of *A muciniphila* daily for 8 weeks	Real-time polymerase chain reaction analysis of fecal	*A muciniphila* protected against atherosclerosis by attenuating the metabolic endotoxemia-induced inflammation, as well as inducing intestinal expression of the tight junction proteins	Li et al. (2016)
IgE-mediated atopic diseases	Atopic (n = 14) and healthy (n = 15) subjects		Fecal microbiota analysis by 454 pyrosequencing	Lower abundance of *Akkermansia* in atopic children	Drell et al. (2015)
Immune-mediated liver injury	Concanavalin A (ConA)-induced immunological liver injury mice model	Oral administration of *A. muciniphila* Muc^T (1.5×10^{10} CFU/mL) for 14 days	Fecal microbiota analyzed by Illumina metagenome sequencing	Negative correlation of *A. muciniphila* with injury-related factors. *A. muciniphila* protected immune-mediated liver injury, alleviated inflammation and hepatocellular death by decreasing proinflammatory cytokines and strengthening intestinal barriers	Wu et al. (2017)
Lipid metabolism	In vitro (organoid culture)	250 µL *A. muciniphila* supernatant		*A. muciniphila* and propionate reduced the risk of gastrointestinal disorders and affected the expression of genes involved in host lipid metabolism and epigenetic activation or silencing of gene expression	Lukovac et al. (2014)
Genetic-induced hyperlipidemia	cAMP-responsive binding protein H (CREBH)–deficient mouse	Oral gavage for 200 µL of *A muciniphila* (2×10^8 CFU/0.2 mL) for 2 weeks		*A. muciniphila* ameliorated chronic hypertriglyceridemia and improved insulin sensitivity	Shen et al. (2016)

However, some studies reported that the higher abundance of *A. muciniphila* in the dextran sodium sulfate (DSS)-induced IBD mice group (Håkansson et al., 2015) and positively interacted with inflammation in murine colitis model (Castro-Mejia et al., 2016). Contradictory to the previous report, Gobert et al. (2016) showed the high prevalence of *A. muciniphila* in the fecal microbiota of constipated-predominant IBS (C-IBS) patient compared to healthy subjects. That exhibited antiinflammatory properties, protected animals from DSS-induced colitis, and alleviated colitis severity in conventional C57BL/6 mice or human microbiota–associated rats along with other gut microbiota members of C-IBS patients (Gobert et al., 2016). Similarly, the administration of *A. muciniphila* strain ATCC BAA-835 showed no signs of intestinal inflammation based on changes in body weight, histopathological scoring, and inflammatory markers in colitis prone gnotobiotic interleukin-10-deficient mice (Ring et al., 2019) as well as reduced chronic low-grade inflammation due to decreased plasma levels of lipopolysaccharide (LPS)-binding protein and leptin, inactivate LPS/and its binding protein downstream signaling in chow diet-fed mice was observed (Zhao et al., 2017).

Numerous human and animal studies showed the abundance of *A. muciniphila* enhanced by several dietary interventions, including FODMAP (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols) diet but decreased with pectin or guar gum (Dao et al., 2016) and even with energy-rich diets such as high fat–diet, high fat high sugar–rich diet, or high fat high cholesterol–rich diet (Zhai, Feng, Arjan, & Chen, 2019). The blooming of *A. muciniphila* by polyphenol-rich foods, including wild blueberry polyphenolic extract (Rodríguez-Daza et al., 2020), cranberry extract (Anhê et al., 2015), nonabsorbable apple procyanidins (Masumoto et al., 2016), grape polyphenols (Roopchand et al., 2015; Zhang et al., 2018), lingonberries (Heyman-Lindén et al., 2016), and pomegranate (Li et al., 2015), influenced intestinal immunomodulatory response and improved metabolic outcomes in high-fat high-sucrose, or high-fat diet-fed mice. Moreover, the abundance of *A. muciniphila* increased by cross-feeding on the products produced by fiber-degrading species, including *Bacteroides ovatus* and *Eubacterium rectale* (Desai et al., 2016), and through calorie restriction in both animal (Sonoyama et al., 2009) and human (Remely et al., 2015) studies. The increased abundance of *A. muciniphila* improved metabolic features in both murine models and human (Wu et al., 2017) in particular, reduced insulin resistance, lipid homeostasis, and adipose fat distribution in overweight and obese subjects (Dao et al., 2016). Furthermore, metformin used to treat metabolic syndromes such as obesity and type 2 diabetes tends to be a growth stimulator for *Akkermansia* spp. (Lee & Ko, 2014). The increased species of this bacteria may grant the antidiabetic effects of metformin (Shin et al., 2014) by influencing the gut microbiota composition (De La Cuesta-Zuluaga et al., 2017).

Such findings offer a basis for developing a therapeutic strategy based on *A. muciniphila* for the diagnosis and intervention of metabolic disorder diseases, including obesity, cardiovascular disease, liver injury, and type 2 diabetes mellitus.

3.1.3 Possible mechanism of Akkermansia muciniphila action

The understanding of the mechanism of action of *A. muciniphila* is important for their new therapeutic possibilities but requires further investigation. This bacterium may share traditional probiotic mechanisms along with its novel mechanism (Fig. 4.2). The mechanism of host regulation by *A. muciniphila* is thought to be through its most abundant outer membrane pili-like, immunomodulatory protein Amuc_1100 (Plovier et al. 2017), its extracellular vesicles (Reunanen et al., 2015), production of essential short-chain fatty acids (SCFAs) (Ottman et al., 2017), secretion of antimicrobial peptides, such as regenerating islet-derived 3-gamma (RegIIIγ) (Everard et al., 2013), stimulation of 2-arachidonoylglycerol (2-AG), and associated 2-oleoylglycerol (2-OG) of the endocannabinoid system (Cani, Geurts, Matamoros, Plovier, & Duparc, 2014).

A. muciniphila and its derived extracellular vesicles strengthen the intestinal barrier by adhering to the intestinal epithelium (Reunanen et al., 2015), increasing the number of goblet cells (Everard et al., 2013) or upregulating the tight junction protein such as occludin, claudins, and zonula occludens expression (Chelakkot et al., 2018). This, in turn, inhibits bacteria-derived LPS penetrating circulation (metabolic endotoxemia) and alleviates inflammation cascade induced by LPS/LPB complex assembling with membrane-bound CD14 (cluster of differentiation 14) molecules and Toll-like receptor 4 (TLR4) on the surface of macrophages through inhibiting nuclear factor-κB (NF-κB) and increased regulatory T (Treg) cells (Li, Lin, Vanhoutte, Woo, & Xu, 2016; Xu et al., 2020). *A. muciniphila* showed antiinflammatory effects through stimulating Toll-like receptor 2 (TLR2) expressing cells and decrease in the expression of TLR4 and proinflammatory cytokines, TNFα and interleukin-6 (IL-6) in obese mice (Ashrafian et al., 2019). Additionally, it decreased the expression of interferon-gamma (IFNγ) that negatively mediated glucose metabolism (Greer et al., 2016) and regulated adaptive and innate immune responses associated with obesity-induced chronic inflammation (Shin et al., 2014). Furthermore, the outer membrane protein Amuc_1100 abled to replicate almost all the effects of live or pasteurized *A. muciniphila* against obesity, insulin resistance, gut barrier alteration (Plovier et al. 2017) and can blunt colitis and colitis-associated colorectal cancer with decreased infiltration of colon macrophages and $CD8^+$ cytotoxic T lymphocytes (Wang et al., 2020).

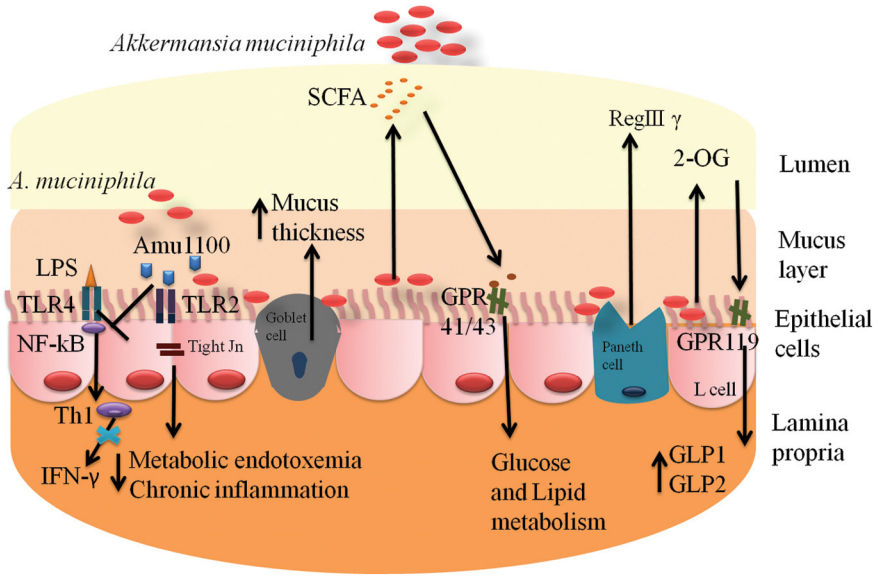


FIGURE 4.2 Schematic diagram summarizing *Akkermansia muciniphila* effect on the host health. The mechanism of host regulation by *A. muciniphila* is thought to be through its outer membrane protein Amuc_1100, production of short-chain fatty acids, antimicrobial peptide RegIIIγ or goblet cells, and stimulating 2-OG in endocannabinoid system. *A. muciniphila* increases the number of mucus-producing goblet cells and tight-junction proteins expressions, thereby maintaining gut barrier. The strengthened gut barrier inhibits bacteria-derived LPS penetrating circulation (metabolic endotoxemia) and alleviating LPS/and its binding protein/TLR4-initiated inflammation cascade through inhibiting NF-κB and increased regulatory T (Treg) cells. *A. muciniphila* increased intestinal levels of 2-AG, and associated 2-OG that stimulates the secretion of GLP-1 and GLP-2 from intestinal L cells by activating GPR119 that regulates glucose homeostasis. Amuc_1100 decreases the expression of TLR4 and activate TLR2 to regulate immune response by decreasing the serum levels of proinflammatory cytokines and chemokines (IL-2, IFNγ, MCP-1, MIP-1a, MIP-1b). The antimicrobial peptide RegIIIγ inhibits pathogenic Gram-positive bacteria. The SCFA such as acetate, propionate, which induce numerous transcription factors such as increase Fiaf production and decrease GPR43, HDAC, and PPARγ expression and stimulate secretion of GLP-1 and GLP-2 from intestinal L cells to control lipid metabolism, glucose homeostasis, immunomodulation, satiety, and obesity. *2-AG*, 2-Arachidonoylglycerol; *2-OG*, 2-oleoylglycerol; *Fiaf*, fasting-induced adipocyte factor; *GLP1/2*, glucagon-like peptides1 and 2; *GPR119*, G protein-coupled receptor119; *GPR43*, G protein coupled receptors; *HDAC*, histone deacetylase; *IFNγ*, interferon-gamma; *IL-2*, interleukin-2; *MCP-1*, monocyte chemoattractant protein-1; *MIP-1a*, macrophage inflammatory proteins alpha; *MIP-1b*, macrophage inflammatory proteins beta; *NF-κB*, nuclear factor-κB; *PPARγ*, peroxisome proliferator-activated receptor gamma; *RegIIIγ*, regenerating islet-derived protein gamma; *TLR*, Toll-like receptor.

Moreover, *A. muciniphila* can produce SCFAs such as acetate, propionate, and to a smaller extent 1,2-propanediol and succinate as a result of mucus degradation (Ottman et al., 2017). These SCFAs activate G protein–coupled receptors (GPR43 by acetate, propionate or GPR41 by other SCFA) which modulate angiopoietin-like protein 4/fasting-induced adipose factor (ANGPTL4/FIAF) that is involved in lipid metabolism in liver and intestinal tissue and prevents inflammation (Ashrafian et al., 2019), histone deacetylases that are associated with various biological processes, including transcriptional regulation of interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) expression, and peroxisome proliferator–activated receptor gamma (PPARγ) that maintains energy homeostasis, modulates inflammatory responses and lipid metabolism in adipose tissue (Lukovac et al., 2014).

Additionally, *A. muciniphila* increased intestinal levels of 2-AG, and associated 2-OG, that is present in the endocannabinoid system, a crucial signaling mechanism for glucose homeostasis and energy metabolism as well as an important target for type 2 diabetes, obesity, and inflammation (Cani et al., 2014; Everard et al., 2013). An increase in the endogenous levels of 2-AG, an agonist of CB1 and CB2 cannabinoid receptor, by inhibiting monoacylglycerol lipase through selective inhibitor protects colitis in mice and reduces metabolic endotoxemia as well as proinflammatory cytokines and peripheral and brain inflammation (Alhouayek, Lambert, Delzenne, Cani, & Muccioli, 2011), whereas 2-OG stimulates the secretion of glucagon-like peptides (GLP-1 and GLP-2) from intestinal L cells by activating G protein–coupled receptor 119 (GPR119) (Hansen et al., 2011) which, in turn, regulates glucose homeostasis and gut barrier function (Everard et al., 2011).

A. muciniphila also increases the intestinal epithelial cells secreted antimicrobial peptides, such as regenerating islet-derived 3-gamma (RegIIIγ) (Everard et al., 2013) to check bacteria and manipulate the composition of the microbiota, particularly Gram-positive species (Zhao et al., 2018) thereby promoting host–bacterial mutualism and regulating the spatial relationships between the microbiota and host (Vaishnava et al., 2011). Although these data indicate the benefits of *A. muciniphila* in host physiological processes, the main underlying mechanisms in those health impacts still to be verified.

3.1.4 Safety aspects of *Akkermansia muciniphila*

Although *A. muciniphila* has several beneficial effects on the host, it is not a general therapy for the disease and can behave as a pathogen in some cases (Routy et al., 2018). Some studies have identified the pathogenic behavior of this bacterium in certain human diseases as mentioned in Table 4.2. *A. muciniphila* can disturb host mucus homeostasis by promoting the

TABLE 4.2 Negative effects of *Akkermansia muciniphila* on health.

Target conditions	Induction type	Analysis type	Findings	References
Salmonella enterica typhimurium–induced gut inflammation	*Salmonella typhimurium*–infected gnotobiotic mice	Quantitative real-time PCR	*A. muciniphila* favors the intestinal growth of *S. enterica* serovar *Typhimurium* (*S. typhimurium*), and impairs *S. typhimurium*–induced intestinal inflammation	Ganesh et al. (2013)
C-IBS patients (n = 33; healthy subjects (n = 58)		Pyrosequencing analysis was performed in fecal samples from C-IBS patients and healthy subjects	Increase relative abundance of *A. muciniphila* in constipated-predominant irritable bowel syndrome C-IBS patients compared to healthy individuals	Gobert et al. (2016)
Colitis	DSS-induced colitis in mice	Quantitative PCR	Higher abundance of *Akkermansia* in colitis group	Håkansson et al. (2015)
Colon tumorigenesis	Intraperitoneal (i.p.) injection with azoxymethane and DSS	454 Titanium sequencing platform	Tumor development correlated with *A. muciniphila* from baseline levels	Zackular et al. (2013)
Colorectal cancer (CRC)	Fecal microbiota transplantation from CRC patients (n = 3) and healthy individuals (n = 3) into germ-free mice as prophylaxis to azoxymethane- and DSS-induced colonic tumor	Illumina MiSeq sequencer	*Akkermansia* positively correlated with increased tumor burden could be a result of their ability to degrade mucin	Baxter, Zackular, Chen, & Schloss (2014)
Colorectal cancer (CRC)	Mutation of the tumor-suppressor gene Apc (FabplCre; Apc15lox/+) induced colorectal cancer mouse model	Shotgun metagenomic sequencing plus quantitative PCR analysis of feces	*A. muciniphila* promoted colorectal tumorigenesis, but if combined with *Helicobacter typhlonius* represses carcinogenesis in FabplCre; Apc (15lox/+) mice	Dingemanse et al. (2015)
Pouchitis	Ulcerative colitis associated pouchitis (n = 9), healthy ulcerative colitis pouches (n = 3), healthy familial adenomatous polyposis pouches (n = 7)	Ileal pouch mucosal biopsies and fecal samples analysis with a 16S rDNA-based terminal restriction fragment length polymorphism (TRFLP) approach	*Akkermansia* significantly more prevalent in pouchitis	Zella et al. (2011)
Graft-vs-host disease (GVHD)	Patients undergoing allogeneic hematopoietic stem cell transplantation (n = 857)	Illumina MiSeq platform	Increase in *Akkermansia* contributed to Graft-vs-host disease may be due to degradation of mucus	Shono et al. (2016)
Type 2 diabetes (T2D)	T2D patients (n-345) and nondiabetic controls	Metagenome-wide association study (MGWAS) based on deep shotgun sequencing of the gut microbial DNA	*A. muciniphila* positively linked with type 2 diabetes samples	Qin et al. (2012)
Parkinson's disease (PD)	Parkinson's disease patients (n = 76), idiopathic rapid eye movement sleep behavior disorder patients (n = 21), and healthy controls (n = 78)	16S and 18S ribosomal RNA amplicon sequencing. HiSeq2500 (Illumina) from stool and nasal wash samples	*Akkermansia* positively linked in stool sample from Parkinson's disease patient but no strong differences in nasal microbiota	Heintz-Buschart et al. (2018)

growth of *Salmonella enterica* serovar *Typhimurium* (*S. typhimurium*), and aggravating the intestinal inflammation induced by *S. typhimurium* (Ganesh, Klopfleisch, Loh, & Blaut, 2013).

Furthermore, *A. muciniphila* strains were found susceptible to some antibiotics, including imipenem, piperacillin/tazobactam, and doxycycline but resistant against vancomycin, metronidazole, and penicillin G in one patient receiving broad-spectrum antibiotic therapy and others only with imipenem (Dubourg et al., 2013). The whole genome sequencing showed *A. muciniphila* as the natural reservoir of some antibiotic resistance genes, including beta-lactamases gene (classes C and A) encoding 5-nitroimidazole antibiotic resistance protein as well as a gene-encoding putative secreted antibiotic biosynthesis. However, the gene related to its pathogenicity was not observed (van Passel et al., 2011).

Even though there is a lack of concrete findings regarding the safety of *A. muciniphila* in humans, some report has suggested that this bacterium should be safe for human treatments. The oral administration of *A. muciniphila* (active or pasteurized) was reported to be safe without any changes in relevant safety clinical parameters and adverse events related to liver, muscles and renal functions as well as markers of immunity and inflammation in obese individuals compared with the healthy control individuals (Plovier et al., 2017; Wang et al., 2020). Altogether, these findings form the basis of investing the role played by *A. muciniphila* as a prognostic and diagnostic tool for human metabolic diseases in the future.

3.2 *Bacteroides fragilis*

Nontoxigenic *B. fragilis* (NTBF) is a beneficial human intestinal commensal that may be used as an emerging NGP for the improvement of inflammation-related diseases, including autoimmune conditions.

3.2.1 Characteristics

B. fragilis is a Gram-negative, obligate anaerobe, rod-shaped bacterium that belongs to phylum Bacteroidetes, class Bacteroidia, order Bacteroidales, and family Bacteroidaceae (Huang, Lee, & Mazmanian, 2011). This bacterium is commonly found on the mucous surface of the GIT but also observed in the mouth, upper respiratory tract, and female genital tract (Kuwahara et al., 2004). Additionally, the ability of *B. fragilis* to produce eight different varieties of capsular polysaccharides on the cell surface (Geva-Zatorsky et al., 2015), metabolize many host and dietary polysaccharides, and withstand oxygen using cytochrome bd oxidase facilitates its effective colonization under harsh intestinal environment (Wexler & Goodman, 2017).

B. fragilis was first isolated from infected patients as a pathogen, primarily named "*Bacillus fragilis* (Wexler & Goodman, 2017), and comprises two subtypes enterotoxigenic *B. fragilis* (ETBF) and NTBF strains. ETBF that possesses *bft* genes encoding *B. fragilis* toxin (BFT) in its pathogenicity islands may disrupt the energy metabolism and cause diarrheal disease, IBD, late-stage colon cancer, and sepsis (Sears, 2009). Conversely, NTBF strains are symbiotic, do not possess any BFT, may antagonize ETBF through interspecific competition and quell colonic inflammation (Hecht et al., 2016), and have been recognized as a candidate of NGPs.

3.2.2 Effects of Bacteroides fragilis in host health

B. fragilis was observed to defend against various pathogens and their associated infection, including *Helicobacter hepaticus* (Mazmanian, Round, & Kasper, 2008), *Bartonella henselae* (Pagliuca et al., 2016; Sommese et al., 2012), *Clostridium perfringens* (Wrigley, 2004), *C. difficile* (Deng et al., 2018), *Vibrio parahaemolyticus* (Li et al., 2017), and *Cronobacter sakazakii* (Fan et al., 2019). *B. fragilis* and its polysaccharide A (PSA) have significant effects on the prevention and treatment of gut-related diseases (Table 4.3) such as colitis (Round & Mazmanian, 2010), *H. hepaticus*–induced colitis (Mazmanian et al., 2008), AAD (Zhang et al., 2018), colorectal cancer (Mehrabian et al., 2018), neurological disorders such as autism spectrum disorder (Hsiao et al., 2013), and multiple sclerosis (Ochoa-Repáraz et al., 2010) by stimulating antiinflammatory cytokine production, strengthening gut barrier integrity and modulating gut microbial composition. These studies have increased the possibility of nonenterotoxigenic *B. fragilis* to be developed as novel potential probiotic therapy.

3.2.3 Possible mechanism of Bacteroides fragilis action

B. fragilis has been shown to modulate host immunological response within the gut and regulate health and disease with the mechanism, including the production of PSA and secretion of several antimicrobial proteins (as you can see in Fig. 4.3). *B. fragilis* produces zwitterionic PSA (Mazmanian et al., 2008) that can be recognized and internalized by antigen presenting cell in the context of major histocompatibility complex class II molecules to induce CD4+ T cell activation unlike other polysaccharides (Mazmanian, Liu, Tzianabos, & Kasper, 2005). PSA induces antiinflammatory cytokine production from Foxp3+ T regulatory cells in the colon to correct the Th 1 cell: Th 2 cell imbalance in the germ-free mice (Round & Mazmanian, 2010) and stimulates interleukin-10-producing CD4+ T cells (T_h cells) and suppresses proinflammatory

TABLE 4.3 Effects of *Bacteroides fragilis* on health.

Target conditions	Study type	Treatment	Findings	References
Necrotizing enterocolitis (NEC)	*Cronobacter sakazakii* induced necrotizing enterocolitis (NEC) in a neonatal rat model	Pretreatment with 0.2 mL formula containing 1×10^9 CFU of *B. fragilis* strain ZY-312	*B. fragilis* ZY-312 suppressed *C. sakazakii*–induced NEC by modulating the proinflammatory response and dual cell death (apoptosis and pyroptosis), as well as reducing epithelial barrier dysfunction, and modulating the compositions of the intestinal bacterial communities	Fan et al. (2019)
Methotrexate induced gastrointestinal toxicity	*Methotrexate intraperitoneally (i.p.) injected mice*	Oral administration of *B. fragilis* (ATCC 25285)	*B. fragilis* ameliorated methotrexate induced inflammatory reactions and modulated macrophage polarization	Zhou et al. (2018)
Inflammation	Intestinal inflammation mice model	Oral administration of *B. fragilis* NCTC 9343 or *B. fragilis* polysaccharide A	*B. fragilis* polysaccharide A protected from inflammatory disease through a functional requirement for interleukin-10-producing CD4+ T cells	Mazmanian et al. (2008)
Colitis	TNBS induced colitis mice model	50 µg of *B. fragilis* polysaccharide A for 6 days	Polysaccharide A of *B. fragilis* prevented and cured colitis	Round and Mazmanian (2010)
Colitis	TLR2 knockout and IL-10 knockout mice	*B. fragilis* outer membrane vesicles (PSA from *B. fragilis* strain NCTC9343)	*B. fragilis* released PSA in outer membrane vesicles (OMVs) that induced immunomodulatory effects and prevented experimental colitis	Shen et al. (2012)
Colitis	Wild-type (WT), TLR4, TLR2, and IL-10 knockout (−/−) germ-free mice	Oral gavage with *B. fragilis* strain NCTC9343 (5×10^7 CFU)	*B. fragilis* prevented acute DSS-induced colitis through the TLR2/IL-10 signal pathway	Chang et al., 2017
Colitis-associated colorectal cancer (CRC)	Dextran sulfate sodium (DSS) and azoxymethane induced colitis-associated colon cancer mice model	*B. fragilis* NCTC9343 or *B. fragilis* polysaccharide A	*B. fragilis* polysaccharide A inhibited gut inflammation and protected colon tumorigenesis by inhibiting expression of C–C chemokine receptor 5 (CCR5) in the gut and inducing Toll-like receptor 2 (TLR2) signaling	Lee et al. (2018)
Colitis-associated colorectal cancer (CRC)	Colitis-associated colorectal cancer patients (n = 93)and healthy individuals (n = 22 with adenoma and n = 27 without adenoma)		*B. fragilis* significantly lowered in the Colitis-associated colorectal cancer group	Ohigashi et al. (2013)
Colorectal cancer	In vitro human colon carcinoma cell lines SW620 and HT29	PSA purified from *B. fragilis* NCTC9343	Polysaccharide A (PSA) from *B. fragilis* induced the production of the pro-inflammatory cytokine, IL-8, but not IL-10, in CRC cells and inhibited CRC cell proliferation by controlling the cell cycle and impaired CRC cell migration and invasion by suppressing epithelial mesenchymal transition	Sittipo et al. (2018)
Clostridium difficile-associated diseases (CDAD)	*C. difficile* infection mouse model	*B. fragilis* strain ZY-312	Abundance of *B. fragilis* decreased in *C. difficile*-associated diseases patients and protected *C. difficile* infection by modulating gut microbiota and alleviating barrier destruction	Deng et al. (2018)

TABLE 4.3 Effects of *Bacteroides fragilis* on health. (*Cont.*)

Target conditions	Study type	Treatment	Findings	References
Antibiotic-associated diarrhea (AAD)	Antibiotic-associated diarrhea (AAD) rat model by exposing rats to appropriate antibiotics	Nontoxic *B. fragilis* strain ZY-312	*B. fragilis* strain ZY-312 ameliorated antibiotic-associated diarrhea symptoms by restoration of intestinal barrier function and enterocyte regeneration in antibiotic-associated diarrhea (AAD) rats	Zhang et al. (2018)
Autism spectrum disorder (ASD)	Maternal immune activation (MIA) mouse model	Oral treatment with *B. fragilis* NCTC 9343(10^{10} CFU) for 6 days	*B. fragilis* modulated maternal immune activation (MIA), altered serum metabolomic profiles, and corrected gut permeability	Hsiao et al. (2013)
Human multiple sclerosis	Experimental autoimmune encephalomyelitis (EAE)	*B. fragilis* (National Collection of Type Culture 9343) polysaccharide A	*B. fragilis* polysaccharide A protected against central nervous system demyelination in experimental autoimmune encephalomyelitis (EAE) by enhancing numbers of IL-10–producing Foxp3^{+}T$_{reg}$ cells	Ochoa-Repáraz et al. (2010)
Asthma	Airway inflammation mice model	Orally gavaged with *B. fragilis* PSA 100 µg/dose	*B. fragilis* PSA protected asthma by activating CD4+Foxp3− T cells in the gut, and resisting unnecessary inflammatory responses via the production of IL-10	Johnson, Jones, & Cobb (2015)
Pulmonary inflammation	Pulmonary inflammation mice model	Orally gavaged with PSA of *B. fragilis*	Polysaccharide A (PSA) from *B. fragilis* prevented the development of lung inflammation by activating cytokine IL-10 production	Johnson, Jones, & Cobb (2018)

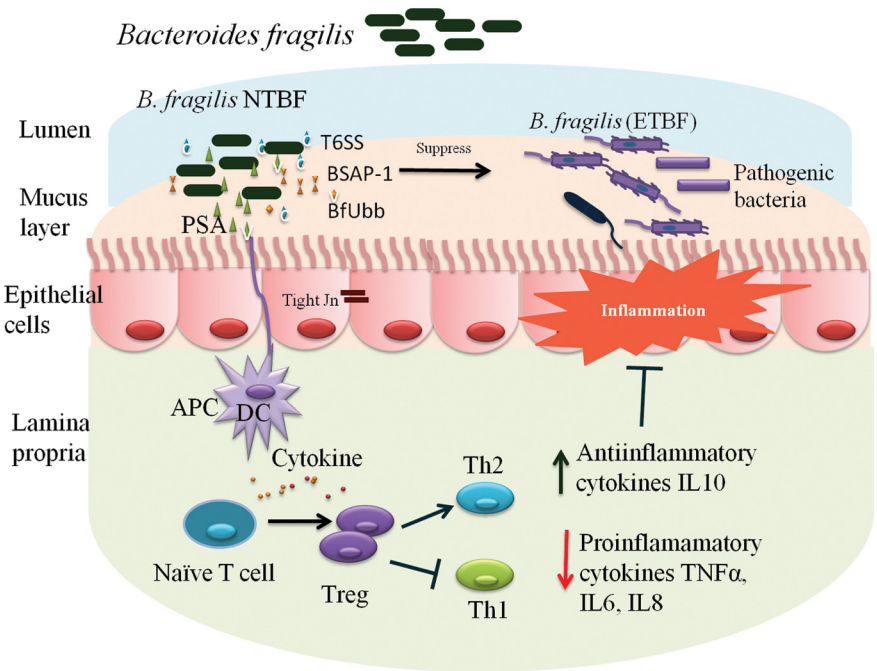


FIGURE 4.3 Schematic diagram summarizing *Bacteroides fragilis* effect on the host health. *B. fragilis* plays a crucial role in modulation of immune system, inflammation, and interbacterial competition by producing unique *PSA* and antimicrobial proteins BSAP-1 and BfUbb, including T6SSs. *PSA* exerts immunomodulation and reduces inflammation through the correction of Th 1 cell: Th 2 cell imbalances, induction of regulatory T cells, stimulation of IL-10 producing CD4+ T cells (Th cells), and suppression proinflammatory IL-17 production. BSAP-1 and BfUbb can lyse bacteria of the same genus via pore formation, while T6SSs inject toxic effector proteins directly into adjacent microbes to inhibit ETBF and other bacterial pathogens. *B. fragilis* also induces the generation of neutrophil extracellular traps and increases SCFA concentration which helps one to reduce inflammation and restore the integrity of the intestinal epithelium. *APC*, Antigen-presenting cell; *BfUbb*, eukaryotic-like ubiquitin protein; *BSAP-1*, Bacteroidales secreted antimicrobial protein-1; *DC*, dendritic cells; *ETBF*, enterotoxigenic *B. fragilis*; *IL-10/IL-17*, interleukin-10/Interleukin-17; *NTBF*, nontoxigenic *B. fragilis; PSA*, *polysaccharide A; T6SSs*, type 4 secretion systems; *Th 1/Th 2 cell*, T helper type 1 and 2; *TNFα*, tumor necrosis factor alpha; *Treg*, regulatory T cells.

interleukin-17 (IL-17) production by intestinal immune cells to reduce inflammation in *H. hepaticus*–induced colitis mouse (Mazmanian et al., 2008). Furthermore, *B. fragilis* secreted outer membrane vesicles (OMVs) containing PSA suppress colitis by activating noncanonical host autophagy, where autophagy-related protein 16-1 (ATG16L1)-deficient dendritic cells (that is often mutated in patients with Crohn's disease) do not induce Treg cell responses upon challenge with OMVs unlike control dendritic cells (Chu et al., 2016). Additionally, *B. fragilis* strains or its polysaccharides effectively promote anticancer immunosurveillance (Routy et al., 2018) and associated with the efficacy of CTLA-4 blockade in anticancer therapy (Vétizou et al., 2015).

B. fragilis secretes antimicrobial proteins called bacteroidales secreted antimicrobial protein-1 (BSAP-1) and eukaryotic-like ubiquitin protein (BfUbb) antagonizing strains of the same genus and type 4 secretion system (T6SS) targeting almost all Bacteroidal organisms, including ETBF (Chatzidaki-Livanis et al., 2017), BSAP-1 contains membrane attack/perforin domains that lyse other bacterial cells via pore formation (Chatzidaki-Livanis, Coyne, & Comstock, 2014), while BfUbb can increase the competitiveness of strains during intraspecies antagonism (Chatzidaki-Livanis et al., 2017). Nevertheless, colonization of *B. fragilis* mainly relies on a tolerant host immune system that allows colonization of the gut, and IgA has even been shown to stabilize the colonization of bacteria (Sun et al., 2019).

Furthermore, *B. fragilis* induces the generation of neutrophil extracellular traps (NETs) and increases SCFA concentration in the luminal microflora of *Salmonella enteritidis* and *S. typhimurium*–infected rats, which helps to reduce inflammation and restore the integrity of the intestinal epithelium (Bukina et al., 2018). Consequently, treatment with *B. fragilis* or its PSA offers an attractive potential approach to modulate host immunological response and other metabolic syndrome.

3.2.4 Safety aspects of Bacteroides fragilis

Commensal NTBF strains provide significant health benefits to the host and have recently been identified as a potential candidate for probiotics to contradict previous studies in which *B. fragilis* was considered as a pathogen (Sun et al., 2019). Some antibiotic, including cefepime, kanamycin, and streptomycin, and drug resistance genes were observed in the chromosome of this bacterium rather than in the plasmid, which decreases the likelihood of spreading resistance (Wang, Yao, Lv, Ling, & Li, 2017). Additionally, *B. fragilis* was found safe and genetically stable in both normal and immune-deficient mice with no risk of transferring antibiotic resistance gene (Wang et al., 2017). However, *B. fragilis* investigation is confined to animal experiment stage, clinical studies are further required to achieve the safety of this organism.

3.3 *Faecalibacterium prausnitzii*

F. prausnitzii decline in intestinal diseases has been documented by growing numbers of studies (Table 4.4) and proposed as sensor and indicator of human health that has sparked interest in considering this bacterium as a new generative probiotic.

3.3.1 Characteristics

F. prausnitzii (formerly *Fusobacterium prausnitzii*) is characterized as Gram-positive, rod-shaped, strictly anaerobic, 47–57 mol% GC content, nonmotile, nonspore forming bacterium that belongs to the phylum Firmicutes, class Clostridia, order Clostridiales, family Ruminococcaceae and is a major bacterium of the *Clostridium leptum* group (Duncan, Hold, Harmsen, Stewart, & Flint, 2002). *F. prausnitzii* is one of the most numerous anaerobic bacteria in the human intestinal microbiota, accounting for around 5%–15% of total detectable bacteria in fecal samples from healthy subjects (Arumugam et al., 2011; Fitzgerald et al., 2018). Its abundance can be explained by its ability to utilize both host and dietary polysaccharides. It feeds on host-derived substrates such as *N*-acetylglucosamine, D-glucosamine and D-glucuronic acid, and β-glucuronidase as well as various dietary polysaccharides, including insoluble carbohydrate such as uronic acids, galacturonic acid but limited to arabinogalactan, xylan, and soluble starch for its growth (Lopez-Siles et al., 2012). The prevalence of *F. prausnitzii* improved with high-fiber (vegetables and fruits) (Ganesan, Chung, Vanamala, & Xu, 2018) and some prebiotic such as galactooligosaccharide (Azcarate-Peril et al., 2017) and xylooligosaccharide (Finegold et al., 2014), while the prevalence decreased with the consumption of higher quantity of animal meat, animal fat, sugar, processed foods, and low-fiber diet (the typical westernized diet) (Ganesan et al., 2018).

F. prausnitzii strains are reported as major butyrate producers in the human intestine (Sokol et al., 2008) and require acetate for the production of butyrate through the butyryl CoA: acetate CoA-transferase pathway (Duncan et al., 2002). Although *F. prausnitzii* strains are not able to use acetate as the sole source of energy upon fermentation of glucose (Duncan et al., 2002), it might be overwhelmed by their cross-feeding metabolic interaction with other commensals at the intestinal mucus layer. The mucin degrading *A. muciniphila* bacterium increases acetate and propionate pool that stimulates syntrophic growth of nonmucus-degrading *F. prausnitzii* and the production of butyrate (Belzer et al., 2017). Similarly, the degradation of nondigestible carbohydrates such as fructooligosaccharides, inulin-type fructans by *Bifidobacterium adolescentis* produces lactate and acetate that is further utilized by *F. prausnitzii* to produce butyrate

TABLE 4.4 Effects of *Faecalibacterium prausnitzii* in health.

Disease	Study type	Treatment	Microbiota analysis technique	Findings	References
Inflammatory bowel disease (IBD)	Healthy controls (17), Crohn's disease patients (20), and Ulcerative colitis patients (22)		Fecal microbiota analysis by PCR targeting 16S rRNA gene sequences and temporal temperature gradient electrophoresis (TTGE)	*F. prausnitzii* reduced in both Crohn's disease and ulcerative colitis patients compared to healthy control	Kabeerdoss, Sankaran, Pugazhendhi, & Ramakrishna (2013)
Inflammatory bowel disease (IBD)	healthy subjects ($n = 32$), patients with IBD ($n = 204$), and other gastrointestinal diseases ($n = 186$)		Fecal microbiota analysis using fluorescence in situ hybridization (FISH)	Depletion of *F. prausnitzii* with a normal leukocyte count in Crohn's disease and increase of leukocytes with higher *F. prausnitzii* in ulcerative colitis patients	Swidsinski, Loening-Baucke, Vaneechoutte, & Doerffel (2008)
Inflammatory bowel disease (IBD)	Active Crohn's disease (CD) patient ($n = 22$), CD patients in remission ($n = 10$), active ulcerative colitis (UC) patient ($n = 13$), UC patients in remission ($n = 4$), infectious colitis (IC) patients ($n = 8$), and healthy subjects ($n = 27$)		Fecal microbiota analysis by quantitative real-time polymerase chain reaction (PCR) targeting the 16S rRNA gene	*F. prausnitzii* species had lower counts in active inflammatory bowel disease and infectious colitis patients compared to healthy subjects	Sokol et al. (2009)
Psoriasis hidradenitis suppurativa and Inflammatory bowel disease (IBD)	Healthy controls ($n = 33$), Psoriasis ($n = 29$), IBD ($n = 31$), and concomitant IBD and psoriasis patient ($n = 13$), and also hidradenitis suppurativa ($n = 17$), and concomitant IBD and HS hidradenitis suppurativa patient ($n = 17$)		Fecal microbiota analysis by quantitative polymerase chain reaction	Lower abundance of *F. prausnitzii* in Psoriasis and IBD patients and no significant difference in *F. prausnitzii* abundance in hidradenitis suppurativa patients	Eppinga et al. (2016)
Inflammatory bowel disease and Colorectal cancer	Healthy controls ($n = 31$), Crohn's disease (CD) patient ($n = 45$), ulcerative colitis (UC) patient ($n = 25$), Irritable bowel syndrome (IBS) patient ($n = 10$), and colorectal cancer patient ($n = 20$)		16S rRNA gene sequencing from biopsies sample	*F. prausnitzii* phylogroup I depleted in Crohn's disease, ulcerative colitis, and colorectal cancer, whereas phylogroup II specifically reduced in Crohn's disease. Lowered levels of both total *F. prausnitzii* and phylogroup I in subjects with Crohn's disease, ulcerative colitis, and colorectal compared with healthy subjects	Lopez-Siles et al. (2016)
Colorectal cancer	Colorectal cancer patient ($n = 20$), patients with upper gastrointestinal cancer ($n = 9$) and healthy volunteers ($n = 17$)		Fecal microbiota analysis by real-time polymerase chain reaction using primers aimed at 16S rDNA	*F. prausnitzii* decreased approximately fourfold in colorectal cancer patients compared to healthy control volunteers	Balamurugan et al. (2008)
Colitis	DNBS-induced colitis mice model	Intragastrically administered 200 µL *F. prausnitzii* strain (1×10^9 CFU) or 200 µL of *F. prausnitzii* strain supernatant for 7 or 10 days		*F. prausnitzii* and its supernatant alleviated the severity of colitis with downregulation of MPO, proinflammatory cytokines, and T-cell levels	Martín et al. (2014)

(*Continued*)

TABLE 4.4 Effects of *Faecalibacterium prausnitzii* in health. (*Cont.*)

Disease	Study type	Treatment	Microbiota analysis technique	Findings	References
Colitis	Dextran sodium sulfate (DSS)-induced colitis mice model	*F. prausnitzii* supernatant		Supernatant of *F. prausnitzii* attenuated the severity of DSS-induced colitis in mice by enhancing the intestinal barrier function	Carlsson et al. (2013)
Irritable bowel syndrome (IBS)	IBS patients (n = 62) and healthy individuals (n = 46)		Phylogenetic microarray in combination with quantitative polymerase chain reaction of fecal sample	Fivefold decreased abundance of *Faecalibacterium* spp. in irritable bowel syndrome (IBS) patients	Rajilić–Stojanović et al. (2011)
Nociceptive in irritable bowel syndrome (IBS)	Neonatal maternal separation (NMS) stress-induced noninflammatory irritable bowel syndrome (IBS)-like rodent model	Intragastrically administered (1 mL of 1×10^9 CFU) *F. prausnitzii* A2-165 or its supernatant	16S rRNA gene sequencing from fecal sample	*F. prausnitzii* exhibited antinociceptive properties in irritable bowel syndrome (IBS) patients with reinforcement of intestinal epithelial barrier	Miquel et al. (2016)
Mucositis	In vitro intestinal cell IEC-6, Caco-2, and T-84 cells and rat model of mucositis treated with 5-fluorouracil (5FU) to induce mucositis	Supernatants derived from *F. prausnitzii* strain		*F. prausnitzii* supernatant reduced the severity of intestinal mucositis in both in vitro and in vivo and partly prevented body weight loss and normalized water intake in mucositis rat model	Wang et al. (2017)
Ulcerative colitis	Ulcerative colitis patients (n = 116) in remission, first-degree relatives (n = 29), and 31 healthy controls (n = 31)		Fecal microbiota analysis by quantitative real-time PCR	Lower abundance of *F. prausnitzii* in ulcerative colitis patients compared to controls	Varela et al. (2013)
Ulcerative colitis	In vitro gut model: M-SHIME (Simulator of the Human Intestinal Microbial Ecosystem) model	Colonization by microbiota from healthy volunteers (n = 6) and UC patients (n = 6)		Lower *F. prausnitzii* in the luminal and mucosal samples from ulcerative colitis patients compared to healthy	Vermeiren et al. (2012)
Ulcerative colitis (UC)	Ulcerative colitis patients (n = 127) and controls		Fecal microbiota analysis using denaturing gradient gel electrophoresis (DGGE) and quantitatively validated using real-time PCR	Reduction of *F. prausnitzii* in UC patients	Machiels et al. (2014)
Crohn's disease (CD)	Crohn's disease patients (n = 161), healthy individuals (n = 121), and inflammatory bowel disease patient (n = 4)		Fecal microbiota analysis by terminal restriction fragment length polymorphism (T-RFLP)	Genus *Faecalibacterium* significantly decreased in Crohn's disease patients with active disease and those in remission as compared with healthy individuals	Andoh et al. (2012)
Crohn's disease	10 monozygotic twin pairs: 2 pairs discordant for predominantly ileal Crohn's disease (ICD), 4 pairs discordant for predominantly colonic Crohn's disease (CCD), 2 pairs concordant for ICD, and 2 pairs concordant for CCD		Microbiota analysis of biopsies samples by terminal-restriction fragment length polymorphism, cloning and sequencing, and quantitative real-time polymerase chain reaction (qPCR)	Lower abundance of *F. prausnitzii* in individuals with predominantly ileal Crohn's disease compared to healthy cotwins and those with Crohn's disease localized in the colon	Willing et al. (2009)

Crohn's disease	Dinitrobenzene sulfonic acid (DNBS)-induced colitis in mice	*Lactococcus lactis*, delivering a plasmid-encoding MAM of *F. prausnitzii* culture supernatants		*F. prausnitzii*–derived antiinflammatory protein inhibited NF-κB pathway in intestinal epithelial cells and prevented colitis in an animal model	Quévrain et al. (2016)
Crohn's disease (CD)	Healthy subjects (n = 15) and Crohn's disease patients (n = 15) subjects. Two patients suffering from ulcerative colitis patients (n = 2) and 1 ischemic colitis patient (n = 1)		Microbiota analysis of fresh biopsy samples obtained from the mucosa by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) of 16S rRNA gene fragments	*Faecalibacterium* positively linked with healthy specimens	Martinez-Medina, Aldeguer, Gonzalez-Huix, Acero, & Garcia-Gil (2006)
Roux-en-Y gastric bypass (RYGB) surgery	Lean control subjects (n = 13) and obese individuals (n = 30) (with seven type 2 diabetics) explored before (M0), 3 months (M3), and 6 months (M6) after RYGB		Fecal microbiota analyzed by real-time quantitative PCR	Lower abundance of *F. prausnitzii* in diabetes and obese and negatively associated with inflammatory markers in obesity and diabetes independently of calorie intake	Furet et al. (2010)
Depression-like and anxiety-like behavior	Chronic unpredictable mild stress–induced depression-like and anxiety-like behavior in rats	*F. prausnitzii* for 4 weeks		*F. prausnitzii* showed anxiolytic and antidepressant-like effects, increased SCFAs and IL-10 levels, and reduced corticosterone C-reaction protein and cytokines interleukin-6 (IL-6) release induced by chronic unpredictable mild stress–induced depression-like and anxiety-like behavior in rats	Hao, Wang, Guo, & Liu (2019)
Diabetes	1645 European women divided into 3 groups: type 2 diabetes, impaired glucose tolerance or normal glucose tolerance		Fecal microbiota analysis by Illumina HiSeq 2000 platform	Lower abundance of *F. prausnitzii* in diabetics compared to nondiabetics	Karlsson et al. (2013)
Type 2 diabetes mellitus	db/db mice (T2DM mouse model)	Supplementation with 1 μg/μL, 200 μL of MAM (microbial antiinflammatory molecule) derived from *F. prausnitzii* and recombinant His-tagged MAM	Abundance of *F. prausnitzii* in the gut microbiota quantified by qRT-PCR	*F. prausnitzii*–derived microbial antiinflammatory molecule (MAM) restored the intestinal barrier structure and function in diabetes mellitus conditions via the regulation of the tight junction pathway	Xu et al. (2020)
Breast cancer	Breast cancer patients (n = 25) and patients with benign breast disease (n = 25)		Fecal microbiota analysis by 16S rDNA sequencing	Abundance of *Faecalibacterium* reduced in breast cancer patients and negatively correlated with various phosphorylcholines. *F. prausnitzii* suppressed the growth of breast cancer cells through the inhibition of IL-6/STAT3 pathway	Ma et al. (2020)
Immuno-modulatory properties	In vitro human and mouse dendritic cells	*F. prausnitzii* A2-165		*F. prausnitzii* A2-165 showed antiinflammatory properties by eliciting high amounts of IL-10 secretion	Rossi et al., 2016
Hepatic health	High fat–fed mice	Intragastrically administration of *F. prausnitzii* (2×10^8 CFU)		*F. prausnitzii* improved hepatic health and decreased adipose tissue inflammation compared to high-fat control mice	Munukka et al. (2017)
Chronic heart failure (CHF)	CHF patients (n = 53) and controls (n = 41)		Metagenomic analyses of faecal samples	*F. prausnitzii* decreased in chronic heart failure (CHF) patients' gut microbiota	Cui et al. (2018)

(Rios-Covian, Gueimonde, Duncan, Flint, & de Los Reyes-Gavilan, 2015; Moens, Weckx, & De Vuyst, 2016) which plays a crucial role in gut physiology.

3.3.2 Effects of Faecalibacterium prausnitzii in host health

F. prausnitzii should be proposed as a sensor and a marker of the human intestinal health (Martín, Bermúdez-Humarán, & Langella, 2018). *F. prausnitzii* or its supernatant has shown a beneficial effect in various metabolic symptoms, including lowering the symptoms of inflammation and improving the intestinal gut barrier in IBD and IBS murine model as well as in clinical trial (Martín et al., 2015; Prosberg, Bendtsen, Vind, Petersen, & Gluud, 2016), and also decreasing insulin resistant, modulating glucose homeostasis (Munukka et al., 2017), and modulating lipid metabolism in HFD mice (Almeida et al., 2019). Additionally, the abundance of *F. prausnitzii* is significantly reduced in patients with metabolic disorders (Table 4.4) such as IBDs (Sokol et al., 2009), Crohn's disease (Andoh et al., 2012), colorectal cancer (CRC) (Lopez-Siles et al., 2016), heart failure (Cui et al., 2018), breast cancer (Ma et al., 2020), diabetes mellitus (Xu et al., 2020), obesity (Furet et al., 2010), and in old-aged individuals (van Tongeren, Slaets, Harmsen, & Welling, 2005).

The depletion of prevalence and abundance of *F. prausnitzii* has been shown in many intestinal disorders, while its depletion in IBD has been reported most extensively (Lopez-Siles et al., 2016). The depletion of phylogroups I and II population of *F. prausnitzii* depends on the condition of the disease. In most intestinal diseases, *F. prausnitzii* phylogroup I is generally depleted, and the phylogroup II numbers are specifically reduced in Crohn's disease. Therefore phylogroup loads can potentially be applied to assist in the diagnosis of gut disease and the classification of IBD location (Lopez-Siles et al., 2016). Similarly, the decreased abundance of *F. prausnitzii* is associated with the inflammatory expression of β-catenin, MMP-9, and NF-κB and damage of epithelial cell that may progress to colorectal cancer (Balamurugan, Rajendiran, George, Samuel, & Ramakrishna, 2008) and might be recognized as useful CRC prognostic biomarkers (Wei et al., 2016), which is similar to Crohn's disease at mucosa level (Lopez-Siles et al., 2016). Collectively, these studies illustrate the enormous potential of *F. prausnitzii* as both a therapeutic target and a diagnostic marker in inflammatory intestinal disorders.

3.3.3 Possible mechanism of Faecalibacterium prausnitzii action

The possible mechanisms behind the benefits of *F. prausnitzii* against gut diseases might be through its extracellular polymeric matrix (EPM) (Rossi et al., 2015), secretion of the microbial antiinflammatory molecule (MAM) bioactive peptides (Quévrain et al., 2016), and production of metabolites, including butyrate and salicylic acid (Miquel et al., 2015) (as you can see in Fig. 4.4). One of the mechanisms used by *F. prausnitzii* to suppress the inflammation is the secretion of bioactive peptides, named MAM (Quévrain et al., 2016). The relative expression of MAM mRNA was statistically lowered in Crohn's disease–active patients and remission compared with healthy control (McLellan et al., 2020). Moreover, *F. prausnitzii* or its supernatant containing MAM attenuated intestinal inflammation and severity of colitis (Breyner et al., 2017). MAM expression exhibited antiinflammatory properties by blocking the NF-κB pathway (Quévrain et al., 2016), and decreasing Th1 and Th17 proinflammatory cytokines, including IL-6, IFNγ (Martín et al., 2014), or IL-17 production (Zhang et al., 2014), and enhancing TGFβ and IL-10 production by peripheral blood mononuclear cell (PBMC), dendritic cells, and macrophages (Sokol et al., 2008). Additionally, MAM restored the intestinal barrier structure and function by regulating the tight junction protein, including upregulation of zona occludens 1 in both in vitro and in vivo diabetes mellitus (*db/db)* murine model (Xu et al., 2020), Claudin-4, and the junctional adhesion molecule F11r in DNBS-treated mice (Martín et al., 2015) or by affecting paracellular permeability in DSS-treated mice (Carlsson et al., 2013).

Furthermore, *F. prausnitzii* prevented breast cancer, maintained gut barrier, and immune function by acting as a major inducer of *Clostridium*-specific IL-10-secreting regulatory, CD4CD8αα (DP8α) T cell (Treg) subset that is present abundantly in the healthy human colonic *lamina propria* and blood compared with IBD patients (Sarrabayrouse, Alameddine, Altare, & Jotereau, 2015). This regulatory T cell (Treg) subset inhibits the secretion of IL-6 cytokine and phosphorylation of Janus kinases 2 (JAK2)/signal transducers while activates transcription 3 (STAT3) in breast cancer cells Ma et al., 2020).

F. prausnitzii produces butyrate by cross-feeding acetate and propionate produced by mucin degrading *A. muciniphila* (Belzer et al., 2017), and also lactate and acetate metabolites produced by nondigestible carbohydrate utilizing *B. adolescentis* (Rios-Covian, Gueimonde, Duncan, Flint, & de Los Reyes-Gavilan, 2015; Moens et al., 2016). Butyrate acts as the main energy source for the epithelial cells and plays a crucial role in maintaining the healthy gut, production of antiinflammatory compounds (Sokol et al., 2008), improving intestinal barrier integrity (Carlsson et al., 2013), and modulating oxidative stress and carcinogenesis (Hamer et al., 2008). Butyrate regulates these activities through inhibiting NF-κB activation and histone deacetylation (Hamer et al., 2008), modulating the activity and expression of the nuclear hormone receptors

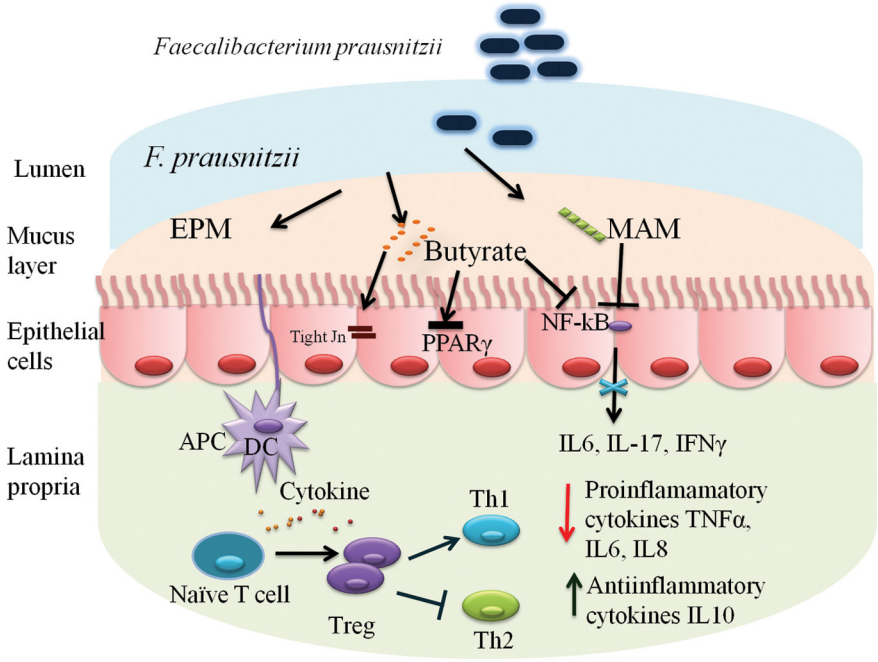


FIGURE 4.4 Schematic diagram summarizing *Faecalibacterium prausnitzii* effect on the host health. *F. prausnitzii* exerts its beneficial impacts on the host by inhibiting inflammation and strengthening the intestinal barrier through its EPM, production of bioactive peptides MAM, butyrate (SCFA), and salicylic acid. MAM can able to enhance TGFβ and IL-10 production and reduce proinflammatory cytokines, including IL6, IL-17, or IFNγ production through inhibiting NF-κB activation as well as strengthening the gut barrier integrity by regulating tight junction protein zona occludens 1 (ZO-1) and Claudin 4. Butyrate inhibits inflammatory response through inhibiting NF-κB activation, histone deacetylation, and IFNγ and upregulating PPARγ. It acts as the primary energy source for the intestinal epithelial cells and maintains intestinal barrier integrity. EPM activates the production of antiinflammatory cytokines, including IL-10 and IL-2 in antigen presenting cells. Salicylic acid is capable of reducing IL-8 by blocking NF-κB. *APC*, Antigen-presenting cell; *DC*, dendritic cells; *EPM*, extracellular polymeric matrix; *IFNγ*, interferon-gamma; *IL-6/IL-8/IL-17/IL-10*, interleukin-6/interleukin-8/ interleukin-17/interleukin-10; *MAM*, microbial antiinflammatory molecule; *NF-κB,* nuclear factor-κB; *PPARγ*, proliferator-activated-receptor γ; *Th1/2,* T helper type 1 and type 2; *TNFα*, tumor necrosis factor alpha; *Treg*, regulatory T cells.

PPARγ and the vitamin D receptor (Schwab et al., 2007) and inhibition of IFNγ/STAT1 (signal transducer and activator of transcription 1) signaling (Klampfer, Huang, Sasazuki, Shirasawa, & Augenlicht, 2003). Martín et al. (2017) reported the butyrate produced by *F. prausnitzii*–induced antiinflammatory cytokine, IL-10, in PBMCs but unable to block IL-8 secretion in TNFα-stimulated HT-29 cells (Martín et al., 2017).

Salicylic acid is another antiinflammatory metabolite produced by *F. prausnitzii* capable of reducing IL-8 by blocking NF-κB and also acts as a precursor of amine derivate 5-aminosalicylic acid (5-ASA or mesalamine) that is used as a drug against IBD patient (Miquel et al., 2015). EPM derived from *F. prausnitzii* strain (HTF-F) has been shown to have an immunomodulatory effect in DSS colitis mouse model through TLR2-dependent modulation of IL-12 and IL-10 cytokine production in antigen-presenting cells (Rossi et al., 2015).

3.3.4 Safety aspects of Faecalibacterium prausnitzii

The pathogenesis of *F. prausnitzii* has not been reported so far in animals or humans (Saarela, 2019). However, the strains of *F. prausnitzii* show large variability in cultural and physiological characteristics, including the production of butyrate, requirement of the growth medium, tolerance to bile salts, and resistance to some antibiotics (Foditsch et al., 2014). The complete genome sequence analysis showed that *F. prausnitzii* genome can harbor gene-encoding resistance against several antibiotics, including beta-lactams, fluoroquinolones, tetracyclines, aminoglycosides, and macrolides (Bag, Ghosh, & Das, 2017). Further research is needed to confirm the characteristics of this bacterium such as natural or acquired-type resistance of antibiotics, and other safety parameters. The safety evaluation of *F. prausnitzii* is limited to a few studies only. Foditsch et al. (2015) reported that the oral and rectal administration of *F. prausnitzii* to neonatal calves is safe and showed neither any adverse effects nor any difference in fecal consistency score, attitude, appetite, or dehydration between the treatment groups (Foditsch et al., 2015). Therefore *F. prausnitzii* could be used as a potential probiotic therapy in human gut diseases.

3.4 *Eubacterium hallii*

E. hallii is another emerging potential candidate for NGP.

3.4.1 Characteristics

E. hallii is a Gram-positive, strictly anaerobic, catalase-negative bacterium that belongs to phylum Firmicutes, class Clostridia, and family Eubacteriaceae (Louis, Young, Holtrop, & Flint, 2010). *E. hallii* is a butyrate-producing gut bacterium falls into Clostridium cluster XIVa (Louis et al., 2010). This bacterium represents about 2%–3% of the total fecal bacteria present in healthy individuals (Louis & Flint, 2009). Its relative abundance can be increased by the intake of fiber-rich dietary molecules such as perennial rhizomatous grass (*Leptochloa chinensis*), fructose, or fructooligosaccharide (Li et al., 2020). However, *E. hallii* is not able to utilize complex oligomers or polymers rather rapidly consumes fermentation metabolites to produce butyrate and propionate (Bunesova, Lacroix, & Schwab, 2018). The growth of *E. hallii* and butyrate production can be stimulated by the presence of metabolic end products such as acetate and lactate produced by inulin-type fructans utilizing lactobacilli and bifidobacteria bacteria. Similarly, other metabolites, including lactose, galactose, and *N*-acetylglucosamine, produced from mucin-degrading *Bifidobacterium bifidum* also promote the growth of *E. hallii* and butyrate production (Bunesova, Lacroix, & Schwab, 2018). Additionally, the ability of *E. hallii* to cross-feed on human milk oligosaccharides fucosyllactose and mucin glycans metabolites produced by other microbes could promote their presence in the infant gut (Bunesova, Lacroix, & Schwab, 2018).

3.4.2 Effects of Eubacterium hallii in host health

The abundance of *E. hallii* is inversely related to certain metabolic diseases (Table 4.5), including Ulcrative colitis and Crohn's disease relative to healthy individuals, illustrating its importance in host health and balanced gut microbiota

TABLE 4.5 Effects of *Eubacterium hallii* on health.

Target conditions	Study type	Treatment	Analysis type	Findings	References
Diabetes	Obese and diabetic *db/db* mice	Oral administration of live or heat-inactivated *E. hallii* (10^8 CFU)		*E. hallii* improved insulin sensitivity and increased energy expenditure in *db/db* mice	Udayappan et al. (2016)
Acute pancreatitis	Patients with different severity of acute pancreatitis (n = 80)		16S rRNA gene sequencing of rectal swab samples	*E. hallii* negatively associated with acute pancreatitis	Yu et al., 2020
Antibiotic exposure	Pregnant women (n = 1172) and information on antibiotic exposure of children (n = 1060)		16S rRNA gene sequencing from stool samples (n = 392) for gut microbiota profiling	*E. hallii* negatively correlated with childhood adiposity, obesity, and repeated exposure to antibiotics	Chen et al., 2020
Pregnancy	Pregnant and nonpregnant C57BL/6 and BALB/c mice		Fecal microbiota composition mouse intestinal tract chip (MITchip)	Higher *E. hallii* in pregnant BALB/c mice compared to other groups	Elderman et al. (2018)
Ulcerative colitis	Ulcerative colitis patients (n = 14) and non-IBD controls (n = 14)		16S ribosomal RNA gene sequences of mucosal biopsies sample	Decreased abundance of *Eubacterium* at the inflamed site of ulcerative colitis patients compared with the corresponding site of non-IBD controls	Hirano et al. (2018)
Crohn's disease	Crohn's disease patient (n = 10) and healthy individual (n = 10)		Fecal microbiota profile using 16S rRNA sequencing	*Eubacterium* significantly decreased in Crohn's disease patients as compared to healthy controls	Takahashi et al. (2016)

(Hirano et al., 2018; Takahashi et al., 2016). *E. hallii* is capable of producing acetate, butyrate, propionate, and formate from glucose and fermentation metabolites, including acetate and DL-lactate in a low pH environment, thereby preventing lactate accumulation in the colonic ecosystem that is linked to various intestinal disorders (Duncan, Louis, & Flint, 2004; Fekry et al. 2016). Moreover, oral administration of live *E. hallii* has been shown to improve glucose homeostasis and enhance energy metabolism in severely obese and diabetic *db/db* mice model (Udayappan et al., 2016).

3.4.3 Possible mechanism of Eubacterium hallii action on host

E. hallii is gaining popularity due to potential to influence host homeostasis as well as intestinal microbiota through their metabolites (as you can see in Fig. 4.5). *E. hallii* produces SCFAs such as butyrate and hydrogen from glucose fermentation along with acetate, butyrate, propionate, and formate from fermentation metabolites of other commensals bacteria. These SCFAs may stimulate the secretion of glucagon-like peptides (GLP-1 and GLP-2) from intestinal L cells (Udayappan et al., 2016) by activating G protein–coupled receptors (GPR43 and GPR41), which strengthens the gut barrier integrity and metabolic homeostasis (Kimura et al., 2013).

This bacterium can metabolize glycerol to 3-hydroxypropionaldehyde (3-HPA, reuterin) that has antimicrobial properties. The conversion of glycerol to 3-HPA involves pduCDE gene–encoded cobalamin-dependent glycerol/diol dehydratase enzyme (Fekry et al. 2016). This enzyme is required for the production of cobalamin, and to utilize 1, 2-propanediol (1,2-PD) to form propionate (Engels, Ruscheweyh, Beerenwinkel, Lacroix, & Schwab, 2016; Schwab et al., 2017). The formation of propionate from 1, 2-PD yields an extra molecule of ATP that might be an advantage (Gänzle, 2015). Additionally, the metabolism of glycerol in *E. hallii* plays an essential role in the conversion of food-derived heterocyclic aromatic amine carcinogen PhIP (2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) to its conjugated metabolite PhIP-M1 (7-hydroxy-5-methyl-3-phenyl-6,7,8,9-tetrahydropyrido[3′,2′:4,5]imidazo [1,2-α]pyrimidin-5-ium chloride) with high efficiency thereby contributing an important role in detoxification of the human colon (Fekry et al., 2016).

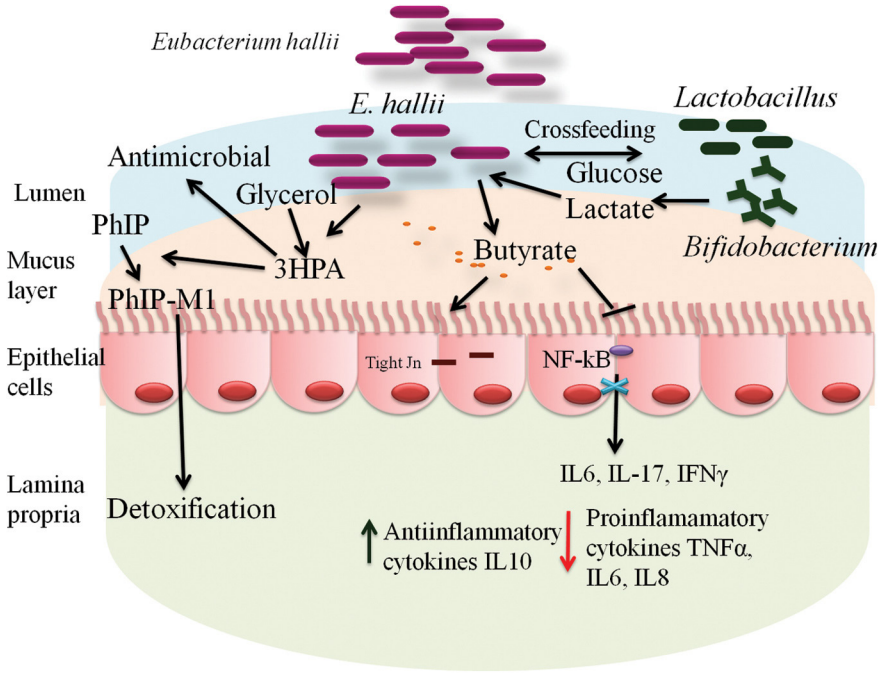


FIGURE 4.5 Schematic diagram summarizing *Eubacterium hallii* effect on the host health. *E. hallii* is an important species among gut microbiota with the potential to impact metabolic equilibrium through the production of various SCFAs, synthesis of glycerol/diol dehydratase enzyme. The SCFAs such as butyrate, acetate, propionate, and formate stimulate the secretion of GLP-1 and GLP-2 from intestinal L cells through the activation of GPR43 and GPR41, which maintains intestinal barrier integrity and metabolic homeostasis. The glycerol/diol dehydratase enzyme helps in the formation of antimicrobial compound 3-HPA (reuterin) from glycerol to act against Gram-positive bacteria. This enzyme also plays a crucial role in converting food carcinogenic heterocyclic amine PhIP to its conjugate PhIP-M1 that has negligible carcinogenic risks, thereby contributing detoxification in the human colon. *SCFAs*, Short-chain fatty acids; *GPR43/41*, G protein coupled receptors; *3-HPA*, 3-hydroxypropionaldehyde; *IL-6/IL-8/IL-17/IL-10*, interleukin-6/interleukin-8/interleukin-17/interleukin-10; *TNFα*, tumor necrosis factor alpha; *IFNγ*, interferon-gamma; *NF-κB*, nuclear factor-κB; *PhIP-M1*, 7-hydroxy-5-methyl-3-phenyl-6,7,8,9-tetrahydropyrido[3′,2′:4,5]imidazo [1,2-α]pyrimidin-5-ium chloride; *GLP-1/2*, glucagon-like peptides 1 and 2; *PhIP*, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine.

3.4.4 Safety aspects of Eubacterium hallii

There are few reports to ensure that *E. hallii* is safe to use without any side effects. Udayappan et al. (2016) reported that the oral administration of *E. hallii* strain even at high dose did not cause any adverse effect in severely obese and diabetic *db/db* mice model compared to the healthy control group. This study supports its safety evidence and emerging potential as a new potential probiotic (Udayappan et al., 2016). Furthermore, more characterization of this bacterium is required to understand the actual beneficial or potential deleterious effects.

3.5 *Parabacteroides goldsteinii*

P. goldsteinii is a commensal that could be a potential novel probiotic candidate for treating obesity.

3.5.1 Characteristics

P. goldsteinii is an obligate anaerobic, 44.6 mol% GC content, nonspore forming, nonmotile, Gram-negative rod-shaped bacterium that belongs to phylum Bacteroidetes, class Bacteroidetes, order Bacteroidales, and family Porphyromonadaceae, which is isolated from human blood (Sakamoto & Benno, 2006; Sakamoto et al., 2009). It was first isolated from the human intestinal sources and identified as *Bacteroides goldsteinii* that was reclassified as *P. goldsteinii* (Sakamoto & Benno, 2006). This bacterium belongs to the human gut microbiota and able to ferment L-arabinose, glucose, lactose, maltose, D-mannose, raffinose, sucrose, and D-xylose, but not cellobiose, glycerol, D-mannitol, melezitose, L-rhamnose, salicin, D-sorbitol, or trehalose (Sakamoto et al., 2009).

3.5.2 Effects of Parabacteroides goldsteinii in host health

The population of P. goldsteinii is negatively correlated with metabolic disorders (Table 4.6) such as obesity (Chang et al., 2015; Wu et al., 2019), chronic kidney diseases (Wu et al., 2020), chronic obstructive pulmonary disease (Wu, 2020), fatty liver disease (Ko et al., 2018), and lung tumors (Wu et al., 2020).

The abundance of *P. goldsteinii* can be affected by dietary interventions that is decreased with high-fat diet whereas increased with some dietary polysaccharide such as high-molecular weight polysaccharides (>300 kDa) fraction of fungus *Hirsutella sinensis* (Wu et al., 2019) and fermented milk kefir (van de Wouw et al., 2020) as well as synbiotic such as a combination of *Lactobacillus gasseri* 505 and *Cudrania tricuspidata* (Oh et al., 2020). The enhanced population of *P. goldsteinii* by fermented milk kefir was associated with *S*-adenosylmethionine synthesis that is used for the treatment of multiple neuropsychiatric conditions (van de Wouw et al., 2020). Wu et al. (2019) reported that the oral administration of *P. goldsteinii* reduced obesity and obesity-associated metabolic disorders, including improved gut barrier, reduced inflammation, and insulin resistance in HFD-fed mice (Wu et al., 2019). Moreover, the increased abundance of *P. goldsteinii* can be used to treat metabolic diseases such as type 2 diabetes, obesity, and fatty liver disease by reducing insulin resistance, improving glucose tolerance (Ko et al., 2018), decreasing body weight, fat accumulation, adipocyte size, and reducing blood aspartate aminotransferase (AST) level, liver weight, hepatic lipid accumulation, and hepatocyte hypertrophy in HFD-fed murine compared to the control (Ko et al., 2018). These studies indicate that *P. goldsteinii* can be used as novel probiotic for treating metabolic disorders.

3.5.3 Possible mechanism of Parabacteroides goldsteinii action

P. goldsteinii reduces inflammation and endotoxemia and helps to maintain intestinal integrity, preserve intestinal homeostasis, and improve gut barrier functions through modulating inflammatory expressions (as you can see in Fig. 4.6). *P. goldsteinii* induced the expression of IL-10 that reduces the inflammation and endotoxemia, maintains the intestinal integrity, and reduces obesity and metabolic disorders in HFD-fed mice (Wu et al., 2019). Additionally, this bacterium reduced the gene expression level of proinflammatory cytokine interleukin-1β (IL-1β)-induced chemokines MCP-1 expression through blocking NF-κB translocation to the nucleus and fibrogenic genes COL3A1, and COL6A1 in kidney tissues. It also enhances the gene expression level of PPARγ coactivator 1α (PGC-1α) which is a key transcriptional regulator of mitochondrial biogenesis and function (Li & Susztak, 2018), PPARγ that is responsible for metabolic changes, including glucose and lipid homeostasis and mitochondrial oxidative metabolism, and acetyl-CoA acyltransferase 2 (Acaa2), carnitine palmitoyltransferase 1 that are known to influence mitochondrial fatty acid β-oxidation (Zhang, Li, Bao, & Huang, 2016) in kidney tissues to effectively improve the activity of the kidney (Wu et al., 2020). Furthermore, it downregulates gene expression level of proinflammatory cytokines IL-1β and TNFα, fibrogenic genes COL3A1, or PGC-1α and enhances the mitochondrial activity of lung cells to repair the mitochondrial dysfunction by upregulating gene expression level of cytochrome *b*, nuclear respiratory factor 1, ribonucleotide reductase RNR1 and RNR2, Sirtuin-1 (SIRT1), and mitochondrial

TABLE 4.6 Effects of *Parabacteroides goldsteinii* on health.

Target conditions	Study type	Treatment	Analysis type	Findings	References
Obesity and type 2 diabetes	High-fat diet (HFD)-fed mice	Oral administration of *P. goldsteinii*	16S rDNA-based microbiota analysis	*P. goldsteinii* negatively linked with high-fat diet Live *P. goldsteinii* prevented body weight gain, improved intestinal integrity, and reduced inflammation and insulin resistance in *Hirsutella sinensis* polysaccharide-treated mice	Wu et al. (2019)
Obesity	Mice fed with high-fat diet	*P. goldsteinii* (2×10^9 CFU/kg of body weight) for 8–16 weeks		*P. goldsteinii* strain inhibited the body weight gain compared with high-fat diet	Wu et al. (2020)
Obesity	Mouse fed with a high-fat diet	*P. goldsteinii* (6.1×10^9 CFU)		*P. goldsteinii* reduced body weight, fat accumulation, and adipocyte size to prevent and treat obesity	Ko et al. (2018)
Obesity	Mice fed a high-fat diet		Gut microbiota analysis from stool sample by 454 FLX pyrosequencer platform	*P. goldsteinii* negatively correlated with obesity	Chang et al. (2015)
Lung cancer	Lung cancer mice	*P. goldsteinii* DSM32939 (1×10^9 CFU)		*P. goldsteinii* protected lung cancer by inhibiting the growth of the lung tumor	Wu et al. (2020)
Chronic kidney disease	High-fat diet-induced chronic kidney disease mice model	Intragastric administration of *P. goldsteinii* (5×10^{10} CFU/kg) for 10 weeks		*P. goldsteinii* protected chronic kidney diseases by improving renal function	Wu et al. (2020)
Fatty liver disease	HFD-fed mice	Intragastric administration of *P. goldsteinii* ATCC strain BAA-1180 (2×10^6 CFU) for 8 weeks		*P. goldsteinii* protected fatty liver disease by reducing blood AST level, liver weight, hepatic lipid accumulation, and hepatocyte hypertrophy	Ko et al. (2018)
Chronic obstructive pulmonary disease	Smoke from twelve 3R4F cigarettes-induced chronic obstruction pulmonary mice model	Oral administration of *P. goldsteinii* (1×10^8 CFU)		*P. goldsteinii* protected chronic obstructive pulmonary disease by improving the lung fibrosis, and the abnormal lung function as well as modulating the expression level of genes involved in the lung fibrosis and the mitochondrial activity in lung tissue	Wu (2020)
Insulin resistance and improve glucose tolerance	HFD-fed mice	Intragastric administration of *P. goldsteinii* ATCC strain (2×10^6 CFU) for 8 weeks		*P. goldsteinii* reduces insulin resistance and improved glucose tolerance	Ko et al. (2018)

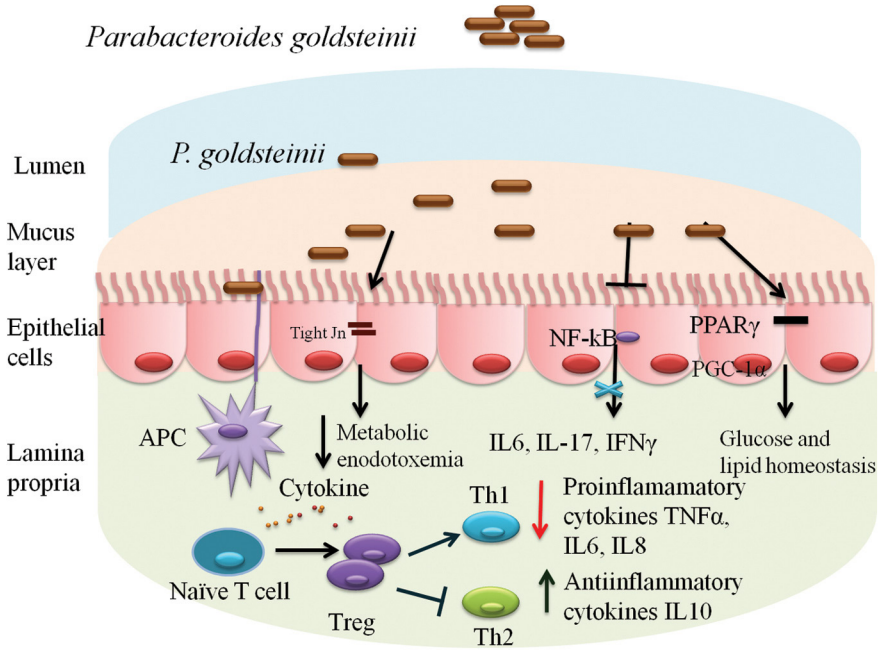


FIGURE 4.6 Schematic diagram summarizing *Parabacteroides goldsteinii* effect on the host health. *P. goldsteinii* reduces inflammation and endotoxemia and helps one to maintain intestinal integrity, preserve intestinal homeostasis and improve gut barrier functions by inducing the expression of IL-10 and decreasing the gene expression level of proinflammatory cytokine interleukin-1β (IL-1β)-induced chemokines Monocyte chemoattractant protein-1 (MCP-1) expression through blocking NF-κB translocation to the nucleus. It ameliorates the symptoms of chronic obstructive pulmonary disease and kidney diseases and maintains metabolic changes, including glucose and lipid homeostasis and mitochondrial oxidative metabolism by enhancing the gene expression level of PGC-1α and PPARγ. *APC*, Antigen-presenting cell; *DC*, dendritic cells; *IFNγ*, interferon-gamma; *IL-6/IL-8/IL-17/IL-10*, interleukin-6/interleukin-8/interleukin-17/interleukin-10; *NF-κB*, nuclear factor-κB; *PGC-1α*, peroxisome proliferator-activated receptor γ coactivator 1α; *PPARγ*, peroxisome proliferator-activated receptor γ; *Th1/2*, T helper type 1 and type 2; *TNFα*, tumor necrosis factor alpha; *Treg*, regulatory T cells.

transcription factor A (TFAM) in chronic obstructive pulmonary to reduce inflammation, lung fibrosis, or oxidative stress in lung tissues (Wu, 2020).

3.5.4 Safety aspects of Parabacteroides goldsteinii

Little research on the health impacts and safety of *P. goldsteinii* has been reported that *P. goldsteinii* can be pathogenic and associated with intraabdominal infection, bacteremia, and septic shock. Its functional characterization indicates that it might be a more common pathogen and has been misidentified in clinical samples due to its phenotype resemblance with *Parabacteroides merdae* and *Parabacteroides distasonis* as well as resistance to some commonly used antimicrobial agents (Awadel-Kariem, Patel, Kapoor, Brazier, & Goldstein, 2010). These safety issues should be thoroughly investigated through whole genome sequence analysis and clinical studies. Therefore more investigations are required to understand the actual beneficial or potential deleterious effects of *P. goldsteinii* before using as therapeutic for metabolic diseases such as obesity.

4 Safety assessment of next-generation probiotics

Unlike traditional *Lactobacillus* and *Bifidobacterium* probiotic species, NGP seems to be more equivocal in terms of their pathogenicity potential (Saarela, 2019). This makes safety assessment as the most obvious concern in identifying the potential NGP candidate. The factors to be considered in the safety assessment of microbes are the distinctive taxonomic classification at the species level, full profiling of strain for the identification of toxin and virulence-related gene, antibiotic resistance and possible horizontal transfer, and other adverse metabolic activity by whole-genome sequence analysis. These genotypic characterizations must be accompanied by advanced safety-focused animal experiments and, ultimately, series of clinical trials (Phase 1 for safety; Phase 2 for efficacy; Phase 3 for effectiveness; and Phase 4 for surveillance) (FAO/WHO, 2002; Saarela, 2019). Besides safety concerns the underlying mechanism on the interactions between the host and probiotics and on how NGP works on targeted diseases needs to be clarified.

The safety issue is one of the major concerns of genetically modified probiotic strain and its use in medical practice. The safety testing should be performed precisely as genetically modified probiotics harbor additional genes responsible for immunomodulation and antigenicity and also capable of affecting the metabolic pathways; therefore the microbe needs to be screened for its virulent traits as well as for their potential pathogenicity (Mazhar et al., 2020). Full safety evaluation is thus the most obvious concern in the characterization of a possible new NGP strain.

5 Application of next-generation probiotics

As the role of intestinal commensals microbiota in the treatment and prevention of multiple disorders is promising, this paves the way for the pharmaceutical and food nutraceutical industries investment.

5.1 Current development

Increasing global awareness of diet-related health issues and mounting evidence of the health benefits of commensal microbes will accelerate market demand for novel probiotics. The commensals can metabolize indigestible compounds and produce certain beneficial compounds such as bioactive peptides, SCFAs, which enables them for future novel food applications in the food industry (Brodmann et al., 2017). Probiotics can be delivered through several food products. Milk and milk products, including yogurt, cheeses, and ice-cream, are good vehicles of probiotic strains due to its inherent unique composition and potential to be stored at refrigerated temperatures as well as provide a suitable environment for the growth and viability of probiotic bacteria. Fruits can provide a suitable medium for the delivery of probiotics, are rich in various nutrients such as minerals, vitamins, dietary fibers, antioxidants, and can be consumed by people having dairy allergens also (Song, Ibrahim, & Hayek, 2012).

Additionally, the commensals secret inhibitory substances or compete with nutrients to prevent the growth of pathogens and maintain gut homeostasis, enabling them to be exploited by pharmaceutical companies as novel disease therapy or live biotherapeutics products (LBP). Nevertheless, a deep safety assessment is required before marketing NGP as LBP (O'Toole et al., 2017). Moreover, commensals have been related to a wide range of human health conditions (Wang et al., 2017). As a result of this linkage, pharmabiotics companies are participating in a total of 650 active research programs in 2020 for microbiome-based therapies in different diseases (Gosálbez, 2020). A few microbiome therapeutic products have completed Phase 3 clinical studies and more are in development stages. SER-109 (Seres Therapeutics) and RBX-2660 (Rebiotix) have completed the Phase 3 trial and primarily suggested for the treatment of *C. difficile* infection (CDI), which is one of the important threats to public health (Moodley & Mistry, 2019). The first microbiome therapeutic product, SER-109, an encapsulated combination of purified probiotic eubacterial spores used to reduce the recurrent CDI, received breakthrough therapy and orphan drug designation from US FDA (Khanna et al., 2016). RBX2660, a suspension of live bacteria, commercially prepared as FMT drug that aims at breaking the cycle of recurrent CDI, received Fast Track, Orphan, and Breakthrough Therapy Status designations from the US FDA (Orenstein et al., 2016; Dubberke et al., 2018). Candidates targeting diseases of the GI tract are Blautix (Blautia hydrogenotrophica; 4D Pharma) for reducing the symptoms of IBS, Thetanix (*Bacteroides thetaiotaomicron*, 4D Pharma) for the treatment of pediatric Crohn's disease have received orphan drug status from the US FDA and SER-287 (Seres Therapeutics) for the reduction of proinflammatory signaling from bacteria for the treatment of ulcerative colitis also look to have good prospects based on published early clinical data (Moodley & Mistry, 2019). Human-derived *Clostridium* strains VE202, generated by Vedanta Biosciences and Johnson and Johnson, was also reported to reverse colitis in murine IBD model by inducing colonic IL-10-producing FOXP3+ regulatory T cells and correction of dysbiosis by reducing the levels of Enterobacteriaceae and Fusobacteria (Oka et al., 2020). Due to the wide scope of microbiome-impacted clinical fields, new microbiome therapies will make a huge difference for many patients across the world, which may be the answer to curing currently incurable diseases.

5.2 Technical challenges

The stability of the probiotic products during its manufacturing and storage process is important, yet several challenges are still unresolved. The viability of the probiotics in sufficient numbers during passage through the GIT along with the selection of optimal culture medium and cell protectants is also important. Microorganisms require complex growth media and strict environmental conditions, including optimum temperature, pH redox potential, and oxygen content (El Hage, Hernandez-Sanabria, & Van de Wiele, 2017). Additionally, they can be mutated and either gain or lose functions upon subculturing; hence, the maintenance of culture in pure form is another challenge.

Maintaining oxygen-free atmospheres and redox potential throughout the manufacturing process and storage is a major hurdle due to the obligate anaerobic nature of these commensal probiotic bacteria. To ensure the absence of oxygen from the processes, several methods have been proposed such as the addition of antioxidants or oxygen scavengers like ascorbic acid (Terpou et al., 2019) or encapsulation of probiotics to maintain its viability and functional ability in harsh environmental conditions (Yao et al., 2020). For instance, the supplementation of flavins, antioxidants such as cysteine or glutathione, and cryoprotectant inulin to the growth medium enables *F. prausnitzii* A2-165 strain to grow in aerobic conditions (Khan et al., 2012; Khan, van Dijl, & Harmsen, 2014). Furthermore, a potentially scalable workflow for the preparation and preservation of high numbers of viable *A. muciniphila* cells under strictly anaerobic conditions has been optimized for therapeutic interventions (Ouwerkerk, Aalvink, Belzer, & De Vos, 2017).

The unique conditions of culture and the complex animal-based medium requirement for the growth and functionality of these commensals as well as enabling safe and cost-effective biomass yield is another challenge. To tackle complex animal-based medium requirements, a human-compatible synthetic medium was developed to promote the growth of *A. muciniphila* with a high yield (Plovier et al., 2017).

Additionally, NGP viability in the harsh condition of the intestinal tract is also essential. The application of sublethal stress (controlled in amplitude and frequency at a given stage of the culture) can be used to enhance microbial robustness and viability. The stressed-induced microbes can be able to cope with industrial methodology and eventually survive in the intestine (Nguyen et al., 2016).

Besides the selection of appropriate delivery vehicles and the mode of therapeutic interventions are equally essential for the use of probiotics in consumer's well-being. However, the main points to be tackled when incorporating probiotics into foods are the selection of food that is compatible with a particular strain growth and viability, the use of probiotic survival compatible food-processing conditions, selection of product matrix, packaging, and environmental conditions to ensure adequate probiotic survival until consumption, and finally also ensure that added strain does not cause any adverse impact on the taste and texture of the product (Nagpal et al., 2012) to ensure efficiency in individual health status (Almeida et al., 2019).

5.3 Regulatory challenges

The existing government regulations for probiotics are neither consistent across all countries nor established on an international basis. Additionally, the claims of probiotics as either dietary supplement or pharmaceutical products (drug) should be considered as there are specific regulatory requirements for each product (Venugopalan, Shriner, & Wong-Beringer, 2010). Nevertheless, various regulatory authorities around the world define and classify probiotics differently.

In the European Union the liable regulatory agency is the European Food Safety Authority (EFSA) that evaluates the safety of foods and ingredients, as well as the scientific validation of health claims. EFSA provides a qualified presumption of safety (QPS) list for the safety evaluation of microorganisms. The traditional probiotic species of *Lactobacillus* and *Bifidobacterium* fall in the QPS list. Additionally, the approval of any microorganism to be used as a drug should be approved by the European Medicines Agency. Similarly, any genetically modified microorganism has to be approved by the EFSA Panel on genetically modified organisms (GMOs) before being marketed. However, it is not clear whether NGP would have to pass through further regulatory investigation or not (O'Toole et al., 2017).

Moreover, in the United States, the liable regulatory agency is the FDA that evaluates a new dietary ingredient (NDI). Instead of using the term "probiotic," their regulatory bodies use the term either "live microbial ingredients" or "live biotherapeutic agents." The term "live microbial ingredients" is used when referring to ingredients in foods or dietary supplements and it should be regulated by the Dietary Supplement Health and Education Act, whereas the term "live biotherapeutic agents" is used when referring to use as a drug and it should be proven to be safe and effective to be approved by the FDA (El Hage et al., 2017). FDA also approves GRAS status for those microorganisms having a long history of safe use. The traditional probiotic species of *Lactobacillus* and *Bifidobacterium* belong to GRAS products and can be exempted from the requirement to notify as NDI. However, when a novel commensal probiotic is proposed to be used as a drug, the product must follow a regulatory process equivalent to that of any new therapeutic product. It must be safe, effective for its intended use, and approved by the FDA before marketing. In contrast to drugs, probiotics to be used as dietary supplements do not need FDA approval but need to notify the FDA before marketing.

Furthermore, the regulatory outlook of a country is constantly changing with the ongoing changes in that nation, which creates significant uncertainty and challenges for the regulatory bodies. Moreover, the status of the probiotic products and how new NGP drug control will comply with LBPs remain unclear. Regulatory frameworks have not addressed microbiome-based therapies and have not cleared through which route these therapies will be assessed (Moodley & Mistry, 2019). Therefore a common regulatory network is needed that will eliminate uncertainty between different regulations and tackle the probiotic issues on an international level.

6 Conclusion

Mounting evidence indicates that the NGPs are promising to treat a variety of health issues with a specific disease that could be more effective than traditional commercial probiotics. Indeed, *A. muciniphila*, *B. fragilis*, *F. prausnitzii*, *E. hallii*, and *P. goldsteinii* are promising NGP candidates that eventually are well connected to general health and can be administered with the intent of enhancing the effectiveness of various chronic diseases. Furthermore, other potential health promoting bacterial strains along with GMOs may also be considered as NGPs candidates. Nonetheless, further work needs to be carried out to better understand the protection, effectiveness, and constraints of the potential NGP and its role in ensuring health and dysbiosis in contributing to the disease, and even the possibility of GM probiotics aimed at clinical conditions requires a robust safety policy to avoid the dissemination of genetic modification into the environment. Additionally, the complexity of the microbiome and its changing nature as well as choosing the right patients for enrollment in clinical trials due to the uniqueness of microbiota within each individual can make not only drug development difficult but diagnosis can also be equally challenging. Moreover, the cost-effective and efficient manufacture of commercial products of live bacteria candidates in oxygen-free atmospheres and complex growth medium requirement is another challenge.

References

Alberti, K. G. M. M., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., et al. (2009). Harmonizing the metabolic syndrome: A joint interim statement of the international diabetes federation task force on epidemiology and prevention; national heart, lung, and blood institute; American heart association; world heart federation; international atherosclerosis society; and international association for the study of obesity. *Circulation*, *120*(16), 1640–1645.

Alhouayek, M., Lambert, D. M., Delzenne, N. M., Cani, P. D., & Muccioli, G. G. (2011). Increasing endogenous 2-arachidonoylglycerol levels counteracts colitis and related systemic inflammation. *The FASEB Journal*, *25*(8), 2711–2721.

Almeida, D., Machado, D., Andrade, J. C., Mendo, S., Gomes, A. M., & Freitas, A. C. (2019). Evolving trends in next-generation probiotics: A 5W1H perspective. *Critical Reviews in Food Science and Nutrition*, *60*(11), 1–14.

Andoh, A., Kuzuoka, H., Tsujikawa, T., Nakamura, S., Hirai, F., Suzuki, Y., et al. (2012). Multicenter analysis of fecal microbiota profiles in Japanese patients with Crohn's disease. *Journal of Gastroenterology*, *47*(12), 1298–1307.

Anhê, F. F., Roy, D., Pilon, G., Dudonné, S., Matamoros, S., Varin, T. V., & Marette, A. (2015). A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased *Akkermansia* spp. population in the gut microbiota of mice. *Gut*, *64*(6), 872–883.

Aron-Wisnewsky, J., Vigliotti, C., Witjes, J., Le, P., Holleboom, A. G., Verheij, J., et al. (2020). Gut microbiota and human NAFLD: Disentangling microbial signatures from metabolic disorders. *Nature Reviews Gastroenterology & Hepatology*, *17*, 1–19.

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D. R., et al. (2011). Enterotypes of the human gut microbiome. *Nature*, *473*(7346), 174–180.

Ashrafian, F., Shahryari, A., Behrouzi, A., Moradi, H. R., Lari, A., Hadifar, S., et al. (2019). Akkermansia muciniphila-derived extracellular vesicles as a mucosal delivery vector for amelioration of obesity in mice. *Frontiers in Microbiology*, *10*, 2155.

Awadel-Kariem, F. M., Patel, P., Kapoor, J., Brazier, J. S., & Goldstein, E. J. (2010). First report of *Parabacteroides goldsteinii* bacteraemia in a patient with complicated intra-abdominal infection. *Anaerobe*, *16*(3), 223–225.

Azcarate-Peril, M. A., Ritter, A. J., Savaiano, D., Monteagudo-Mera, A., Anderson, C., Magness, S. T., et al. (2017). Impact of short-chain galactooligosaccharides on the gut microbiome of lactose-intolerant individuals. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(3), E367–E375.

Bag, S., Ghosh, T. S., & Das, B. (2017). Complete genome sequence of *Faecalibacterium prausnitzii* isolated from the gut of a healthy Indian adult. *Genome Announcements*, *5*(46), e01286-17.

Balamurugan, R., Rajendiran, E., George, S., Samuel, G. V., & Ramakrishna, B. S. (2008). Real-time polymerase chain reaction quantification of specific butyrate-producing bacteria, *Desulfovibrio* and *Enterococcus faecalis* in the feces of patients with colorectal cancer. *Journal of Gastroenterology and Hepatology*, *23*(8 Pt 1), 1298–1303.

Bäumler, A. J., & Sperandio, V. (2016). Interactions between the microbiota and pathogenic bacteria in the gut. *Nature*, *535*(7610), 85–93.

Baxter, N. T., Zackular, J. P., Chen, G. Y., & Schloss, P. D. (2014). Structure of the gut microbiome following colonization with human feces determines colonic tumor burden. *Microbiome*, *2*(1), 20.

Belkaid, Y., & Naik, S. (2013). Compartmentalized and systemic control of tissue immunity by commensals. *Nature Immunology*, *14*(7), 646–653.

Belzer, C., & De Vos, W. M. (2012). Microbes inside—From diversity to function: The case of *Akkermansia*. *The ISME Journal*, *6*(8), 1449–1458.

Belzer, C., Chia, L. W., Aalvink, S., Chamlagain, B., Piironen, V., Knol, J., et al. (2017). Microbial metabolic networks at the mucus layer lead to diet-independent butyrate and vitamin B12 production by intestinal symbionts. *mBio*, *8*(5), e00770-17.

Breyner, N. M., Michon, C., de Sousa, C. S., Vilas Boas, P. B., Chain, F., Azevedo, V. A., et al. (2017). Microbial anti-inflammatory molecule (MAM) from *Faecalibacterium prausnitzii* shows a protective effect on DNBS and DSS-induced colitis model in mice through inhibition of NF-κB pathway. *Frontiers in Microbiology*, *8*, 114.

Brodmann, T., Endo, A., Gueimonde, M., Vinderola, G., Kneifel, W., de Vos, W. M., et al. (2017). Safety of novel microbes for human consumption: Practical examples of assessment in the European Union. *Frontiers in Microbiology*, *8*, 1725.

Bukina, Y. V., Varynskyi, B. O., Voitovich, A. V., Koval, G. D., Kaplaushenko, A. G., & Kamyshnyi, O. M. (2018). The definition of neutrophil extracellular traps and the concentration of short-chain fatty acids in salmonella-induced inflammation of the intestine against the background of vancomycin and Bacteroides fragilis. *Microbila Ecology*, *75*, 228–238.

Bunesova, V., Lacroix, C., & Schwab, C. (2018). Mucin cross-feeding of infant bifidobacteria and *Eubacterium hallii*. *Microbial Ecology*, *75*(1), 228–238.

Cani, P. D., Geurts, L., Matamoros, S., Plovier, H., & Duparc, T. (2014). Glucose metabolism: Focus on gut microbiota, the endocannabinoid system and beyond. *Diabetes & Metabolism*, *40*(4), 246–257.

Carlsson, A. H., Yakymenko, O., Olivier, I., Håkansson, F., Postma, E., Keita, Å. V., et al. (2013). *Faecalibacterium prausnitzii* supernatant improves intestinal barrier function in mice DSS colitis. *Scandinavian Journal of Gastroenterology*, *48*(10), 1136–1144.

Castro-Mejia, J., Jakesevic, M., Krych, Ł., Nielsen, D. S., Hansen, L. H., Sondergaard, B. C., et al. (2016). Treatment with a monoclonal anti-IL-12p40 antibody induces substantial gut microbiota changes in an experimental colitis model. *Gastroenterology Research and Practice*, *2016*, 1–12.

Chang, C. J., Lin, C. S., Lu, C. C., Martel, J., Ko, Y. F., Ojcius, D. M., et al. (2015). *Ganoderma lucidum* reduces obesity in mice by modulating the composition of the gut microbiota. *Nature Communications*, *6*(1), 1–19.

Chang, Y. C., Ching, Y. H., Chiu, C. C., Liu, J. Y., Hung, S. W., Huang, W. C., et al. (2017). TLR2 and interleukin-10 are involved in Bacteroides fragilis-mediated prevention of DSS-induced colitis in gnotobiotic mice. *PLoS One*, *12*(7), 1–16.

Chatzidaki-Livanis, M., Coyne, M. J., & Comstock, L. E. (2014). An antimicrobial protein of the gut symbiont *Bacteroides fragilis* with a MACPF domain of host immune proteins. *Molecular Microbiology*, *94*(6), 1361–1374.

Chatzidaki-Livanis, M., Coyne, M. J., Roelofs, K. G., Gentyala, R. R., Caldwell, J. M., & Comstock, L. E. (2017). Gut symbiont Bacteroides fragilis secretes a eukaryotic-like ubiquitin protein that mediates intraspecies antagonism. *mBio*, *8*(6), e01902-17.

Chelakkot, C., Choi, Y., Kim, D. K., Park, H. T., Ghim, J., Kwon, Y., et al. (2018). *Akkermansia muciniphila*-derived extracellular vesicles influence gut permeability through the regulation of tight junctions. *Experimental & Molecular Medicine*, *50*(2), e450.

Chen, L. W., Xu, J., Soh, S. E., Aris, I. M., Tint, M. T., Gluckman, P. D., et al. (2020). Implication of gut microbiota in the association between infant antibiotic exposure and childhood obesity and adiposity accumulation. *International Journal of Obesity*, *44*, 1–13.

Chu, H., Khosravi, A., Kusumawardhani, I. P., Kwon, A. H., Vasconcelos, A. C., Cunha, L. D., et al. (2016). Gene-microbiota interactions contribute to the pathogenesis of inflammatory bowel disease. *Science*, *352*(6289), 1116–1120.

Collado, M. C., Derrien, M., Isolauri, E., de Vos, W. M., & Salminen, S. (2007). Intestinal integrity and *Akkermansia muciniphila*, a mucin-degrading member of the intestinal microbiota present in infants, adults, and the elderly. *Applied and Environment Microbiology*, *73*(23), 7767–7770.

Crovesy, L., Masterson, D., & Rosado, E. L. (2020). Profile of the gut microbiota of adults with obesity: A systematic review. *European Journal of Clinical Nutrition*, *74*, 1–12.

Cui, X., Ye, L., Li, J., Jin, L., Wang, W., Li, S., et al. (2018). Metagenomic and metabolomic analyses unveil dysbiosis of gut microbiota in chronic heart failure patients. *Scientific Reports*, *8*(1), 1–15.

Dao, M. C., Everard, A., Aron-Wisnewsky, J., Sokolovska, N., Prifti, E., Verger, E. O., et al. (2016). *Akkermansia muciniphila* and improved metabolic health during a dietary intervention in obesity: Relationship with gut microbiome richness and ecology. *Gut*, *65*(3), 426–436.

De La Cuesta-Zuluaga, J., Mueller, N. T., Corrales-Agudelo, V., Velásquez-Mejía, E. P., Carmona, J. A., Abad, J. M., et al. (2017). Metformin is associated with higher relative abundance of mucin-degrading *Akkermansia muciniphila* and several short-chain fatty acid–producing microbiota in the gut. *Diabetes Care*, *40*(1), 54–62.

Deng, H., Yang, S., Zhang, Y., Qian, K., Zhang, Z., Liu, Y., et al. (2018). *Bacteroides fragilis* prevents *Clostridium difficile* infection in a mouse model by restoring gut barrier and microbiome regulation. *Frontiers in Microbiology*, *9*, 2976.

Depommier, C., Everard, A., Druart, C., Plovier, H., Van Hul, M., Vieira-Silva, S., et al. (2019). Supplementation with *Akkermansia muciniphila* in overweight and obese human volunteers: A proof-of-concept exploratory study. *Nature Medicine*, *25*(7), 1096–1103.

Depommier, C., Van Hul, M., Everard, A., Delzenne, N. M., De Vos, W. M., & Cani, P. D. (2020). Pasteurized *Akkermansia muciniphila* increases whole-body energy expenditure and fecal energy excretion in diet-induced obese mice. *Gut Microbes*, *11*(5), 1–15.

Derrien, M., Collado, M. C., Ben-Amor, K., Salminen, S., & de Vos, W. M. (2008). The mucin degrader *Akkermansia muciniphila* is an abundant resident of the human intestinal tract. *Applied and Environment Microbiology*, *74*(5), 1646–1648.

Derrien, M., Vaughan, E. E., Plugge, C. M., & de Vos, W. M. (2004). *Akkermansia muciniphila* gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. *International Journal of Systematic and Evolutionary Microbiology*, *54*(5), 1469–1476.

Desai, M. S., Seekatz, A. M., Koropatkin, N. M., Kamada, N., Hickey, C. A., Wolter, M., et al. (2016). A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell*, *167*(5), 1339–1353.

Dingemanse, C., Belzer, C., Van Hijum, S. A., Günthel, M., Salvatori, D., den Dunnen, J. T., et al. (2015). *Akkermansia muciniphila* and *Helicobacter typhlonius* modulate intestinal tumor development in mice. *Carcinogenesis*, *36*(11), 1388–1396.

Doron, S., & Snydman, D. R. (2015). Risk and safety of probiotics. *Clinical Infectious Diseases*, *60*(Suppl. 2), S129–S134.

Drell, T., Larionova, A., Voor, T., Simm, J., Julge, K., Heilman, K., et al. (2015). Differences in gut microbiota between atopic and healthy children. *Current Microbiology*, *71*(2), 177–183.

Dubberke, E. R., Lee, C. H., Orenstein, R., Khanna, S., Hecht, G., & Gerding, D. N. (2018). Results from a randomized, placebo-controlled clinical trial of a RBX2660—A microbiota-based drug for the prevention of recurrent *Clostridium difficile* infection. *Clinical Infectious Diseases*, *67*(8), 1198–1204.

Dubourg, G., Lagier, J. C., Armougom, F., Robert, C., Audoly, G., Papazian, L., et al. (2013). High-level colonisation of the human gut by Verrucomicrobia following broad-spectrum antibiotic treatment. *International Journal of Antimicrobial Agents*, *41*(2), 149–155.

Duncan, S. H., Hold, G. L., Harmsen, H. J., Stewart, C. S., & Flint, H. J. (2002). Growth requirements and fermentation products of *Fusobacterium prausnitzii*, and a proposal to reclassify it as *Faecalibacterium prausnitzii* gen. nov., comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, *52*(6), 2141–2146.

Duncan, S. H., Louis, P., & Flint, H. J. (2004). Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. *Applied and Environment Microbiology*, *70*(10), 5810–5817.

Earley, H., Lennon, G., Balfe, Á., Coffey, J. C., Winter, D. C., & O'Connell, P. R. (2019). The abundance of *Akkermansia muciniphila* and its relationship with sulphated colonic mucins in health and ulcerative colitis. *Scientific Reports*, *9*(1), 1–9.

El Hage, R., Hernandez-Sanabria, E., & Van de Wiele, T. (2017). Emerging trends in "smart probiotics": Functional consideration for the development of novel health and industrial applications. *Frontiers in Microbiology*, *8*, 1889.

Elderman, M., Hugenholtz, F., Belzer, C., Boekschoten, M., de Haan, B., de Vos, P., et al. (2018). Changes in intestinal gene expression and microbiota composition during late pregnancy are mouse strain dependent. *Scientific Reports*, *8*(1), 1–12.

Engels, C., Ruscheweyh, H. J., Beerenwinkel, N., Lacroix, C., & Schwab, C. (2016). The common gut microbe *Eubacterium hallii* also contributes to intestinal propionate formation. *Frontiers in Microbiology*, *7*, 713.

Eppinga, H., Sperna Weiland, C. J., Thio, H. B., van der Woude, C. J., Nijsten, T. E., Peppelenbosch, M. P., et al. (2016). Similar depletion of protective *Faecalibacterium prausnitzii* in psoriasis and inflammatory bowel disease, but not in hidradenitis suppurativa. *Journal of Crohn's and Colitis*, *10*(9), 1067–1075.

Everard, A., Belzer, C., Geurts, L., Ouwerkerk, J. P., Druart, C., Bindels, L. B., et al. (2013). Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(22), 9066–9071.

Everard, A., Lazarevic, V., Derrien, M., Girard, M., Muccioli, G. G., Neyrinck, A. M., et al. (2011). Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. *Diabetes*, *60*(11), 2775–2786.

Fan, H., Chen, Z., Lin, R., Liu, Y., Wu, X., Puthiyakunnon, S., et al. (2019). *Bacteroides fragilis* strain ZY-312 defense against *Cronobacter sakazakii*-induced necrotizing enterocolitis in vitro and in a neonatal rat model. *mSystems*, *4*(4), e00305-19.

Fekry, M. I., Engels, C., Zhang, J., Schwab, C., Lacroix, C., Sturla, S. J., et al. (2016). The strict anaerobic gut microbe *Eubacterium hallii* transforms the carcinogenic dietary heterocyclic amine 2-amino-1-methyl-6-phenylimidazo [4, 5-b] pyridine (PhIP). *Environmental Microbiology Reports*, *8*(2), 201–209.

Finegold, S. M., Li, Z., Summanen, P. H., Downes, J., Thames, G., Corbett, K., et al. (2014). Xylooligosaccharide increases bifidobacteria but not lactobacilli in human gut microbiota. *Food & Function*, *5*(3), 436–445.

Fitzgerald, C. B., Shkoporov, A. N., Sutton, T. D., Chaplin, A. V., Velayudhan, V., Ross, R. P., et al. (2018). Comparative analysis of *Faecalibacterium prausnitzii* genomes shows a high level of genome plasticity and warrants separation into new species-level taxa. *BMC Genomics*, *19*(1), 931.

Foditsch, C., Pereira, R. V. V., Ganda, E. K., Gomez, M. S., Marques, E. C., Santin, T., et al. (2015). Oral administration of *Faecalibacterium prausnitzii* decreased the incidence of severe diarrhea and related mortality rate and increased weight gain in preweaned dairy heifers. *PLoS One*, *10*(12), 1–17.

Foditsch, C., Santos, T. M., Teixeira, A. G., Pereira, R. V., Dias, J. M., Gaeta, N., et al. (2014). Isolation and characterization of *Faecalibacterium prausnitzii* from calves and piglets. *PLoS One*, *9*(12), 1–19.

Furet, J. P., Kong, L. C., Tap, J., Poitou, C., Basdevant, A., Bouillot, J. L., et al. (2010). Differential adaptation of human gut microbiota to bariatric surgery–induced weight loss: Links with metabolic and low-grade inflammation markers. *Diabetes*, *59*(12), 3049–3057.

Ganesan, K., Chung, S. K., Vanamala, J., & Xu, B. (2018). Causal relationship between diet-induced gut microbiota changes and diabetes: A novel strategy to transplant *Faecalibacterium prausnitzii* in preventing diabetes. *International Journal of Molecular Sciences*, *19*(12), 3720.

Ganesh, B. P., Klopfleisch, R., Loh, G., & Blaut, M. (2013). Commensal *Akkermansia muciniphila* exacerbates gut inflammation in *Salmonella typhimurium*-infected gnotobiotic mice. *PLoS One*, *8*(9), e74963.

Gänzle, M. G. (2015). Lactic metabolism revisited: Metabolism of lactic acid bacteria in food fermentations and food spoilage. *Current Opinion in Food Science*, *2*, 106–117.

Geerlings, S. Y., Kostopoulos, I., De Vos, W. M., & Belzer, C. (2018). *Akkermansia muciniphila* in the human gastrointestinal tract: When, where, and how? *Microorganisms*, *6*(3), 75.

Gensollen, T., Iyer, S. S., Kasper, D. L., & Blumberg, R. S. (2016). How colonization by microbiota in early life shapes the immune system. *Science*, *352*(6285), 539–544.

Geva-Zatorsky, N., Alvarez, D., Hudak, J. E., Reading, N. C., Erturk-Hasdemir, D., Dasgupta, S., et al. (2015). In vivo imaging and tracking of host–microbiota interactions via metabolic labeling of gut anaerobic bacteria. *Nature Medicine*, *21*(9), 1091.

Gobert, A. P., Sagrestani, G., Delmas, E., Wilson, K. T., Verriere, T. G., Dapoigny, M., et al. (2016). The human intestinal microbiota of constipated-predominant irritable bowel syndrome patients exhibits anti-inflammatory properties. *Scientific Reports*, *6*(1), 1–12.

Gómez-Gallego, C., Pohl, S., Salminen, S., De Vos, W. M., & Kneifel, W. (2016). *Akkermansia muciniphila*: A novel functional microbe with probiotic properties. *Beneficial Microbes*, *7*(4), 571–584.

Gosálbez, L. (2020). *The microbiome biotech landscape: An analysis of the pharmaceutical pipeline*. .

Greer, R. L., Dong, X., Moraes, A. C. F., Zielke, R. A., Fernandes, G. R., Peremyslova, E., et al. (2016). *Akkermansia muciniphila* mediates negative effects of IFNγ on glucose metabolism. *Nature Communications*, *7*(1), 1–13.

Håkansson, Å., Tormo-Badia, N., Baridi, A., Xu, J., Molin, G., Hagslätt, M. L., et al. (2015). Immunological alteration and changes of gut microbiota after dextran sulfate sodium (DSS) administration in mice. *Clinical and Experimental Medicine*, *15*(1), 107–120.

Hamer, H. M., Jonkers, D. M. A. E., Venema, K., Vanhoutvin, S. A. L. W., Troost, F. J., & Brummer, R. J. (2008). The role of butyrate on colonic function. *Alimentary Pharmacology & Therapeutics*, *27*(2), 104–119.

Hansen, K. B., Rosenkilde, M. M., Knop, F. K., Wellner, N., Diep, T. A., Rehfeld, J. F., et al. (2011). 2-Oleoyl glycerol is a GPR119 agonist and signals GLP-1 release in humans. *The Journal of Clinical Endocrinology & Metabolism*, *96*(9), E1409–E1417.

Hao, Z., Wang, W., Guo, R., & Liu, H. (2019). *Faecalibacterium prausnitzii* (ATCC 27766) has preventive and therapeutic effects on chronic unpredictable mild stress-induced depression-like and anxiety-like behavior in rats. *Psychoneuroendocrinology*, *104*, 132–142.

Hasan, N., & Yang, H. (2019). Factors affecting the composition of the gut microbiota, and its modulation. *PeerJ*, *7*, e7502.

Hecht, A. L., Casterline, B. W., Earley, Z. M., Goo, Y. A., Goodlett, D. R., & Wardenburg, J. B. (2016). Strain competition restricts colonization of an enteric pathogen and prevents colitis. *EMBO Reports*, *17*(9), 1281–1291.

Heintz-Buschart, A., Pandey, U., Wicke, T., Sixel-Döring, F., Janzen, A., Sittig-Wiegand, E., et al. (2018). The nasal and gut microbiome in Parkinson's disease and idiopathic rapid eye movement sleep behavior disorder. *Movement Disorders*, *33*(1), 88–98.

Heyman-Lindén, L., Kotowska, D., Sand, E., Bjursell, M., Plaza, M., Turner, C., et al. (2016). Lingonberries alter the gut microbiota and prevent low-grade inflammation in high-fat diet fed mice. *Food & Nutrition Research*, *60*(1), 29993.

Hirano, A., Umeno, J., Okamoto, Y., Shibata, H., Ogura, Y., Moriyama, T., et al. (2018). Comparison of the microbial community structure between inflamed and non-inflamed sites in patients with ulcerative colitis. *Journal of Gastroenterology and Hepatology*, *33*(9), 1590–1597.

Hsiao, E. Y., McBride, S. W., Hsien, S., Sharon, G., Hyde, E. R., McCue, T., et al. (2013). Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell*, *155*(7), 1451–1463.

Huang, J. Y., Lee, S. M., & Mazmanian, S. K. (2011). The human commensal *Bacteroides fragilis* binds intestinal mucin. *Anaerobe*, *17*(4), 137–141.

Huynh, K., Schneider, M., & Gareau, M. G. (2016). Altering the gut microbiome for cognitive benefit? In *The gut-brain axis* (pp. 319–337). Amsterdam, The Netherlands: Elsevier.

Johnson, J. L., Jones, M. B., & Cobb, B. A. (2015). Bacterial capsular polysaccharide prevents the onset of asthma through T-cell activation. *Glycobiology*, *25*(4), 368–375.

Johnson, J. L., Jones, M. B., & Cobb, B. A. (2018). Polysaccharide-experienced effector T cells induce IL-10 in FoxP3+ regulatory T cells to prevent pulmonary inflammation. *Glycobiology*, *28*(1), 50–58.

FAO/WHO. (2002). In *Joint Food and Agriculture Organization of the United Nations/World Health Organization Working Group report on drafting guidelines for the evaluation of probiotics in food*London, Ontario, Canada: FAO/WHO.

Joyce, S. A., & Gahan, C. G. (2014). The gut microbiota and the metabolic health of the host. *Current Opinion in Gastroenterology*, *30*(2), 120–127.

Kabeerdoss, J., Sankaran, V., Pugazhendhi, S., & Ramakrishna, B. S. (2013). *Clostridium leptum* group bacteria abundance and diversity in the fecal microbiota of patients with inflammatory bowel disease: A case–control study in India. *BMC Gastroenterology*, *13*(1), 20.

Kang, C. S., Ban, M., Choi, E. J., Moon, H. G., Jeon, J. S., Kim, D. K., et al. (2013). Extracellular vesicles derived from gut microbiota, especially *Akkermansia muciniphila*, protect the progression of dextran sulfate sodium-induced colitis. *PLoS One*, *8*(10), 1–11.

Karlsson, C. L., Önnerfält, J., Xu, J., Molin, G., Ahrné, S., & Thorngren-Jerneck, K. (2012). The microbiota of the gut in preschool children with normal and excessive body weight. *Obesity*, *20*(11), 2257–2261.

Karlsson, F. H., Tremaroli, V., Nookaew, I., Bergström, G., Behre, C. J., Fagerberg, B., et al. (2013). Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature*, *498*(7452), 99–103.

Khan, M. T., Duncan, S. H., Stams, A. J., Van Dijl, J. M., Flint, H. J., & Harmsen, H. J. (2012). The gut anaerobe *Faecalibacterium prausnitzii* uses an extracellular electron shuttle to grow at oxic–anoxic interphases. *The ISME Journal*, *6*(8), 1578–1585.

Khan, M. T., van Dijl, J. M., & Harmsen, H. J. (2014). Antioxidants keep the potentially probiotic but highly oxygen-sensitive human gut bacterium *Faecalibacterium prausnitzii* alive at ambient air. *PLoS One*, *9*(5), 1–7.

Khanna, S., Pardi, D. S., Kelly, C. R., Kraft, C. S., Dhere, T., Henn, M. R., et al. (2016). A novel microbiome therapeutic increases gut microbial diversity and prevents recurrent *Clostridium difficile* infection. *The Journal of Infectious Diseases*, *214*(2), 173–181.

Kimura, I., Ozawa, K., Inoue, D., Imamura, T., Kimura, K., Maeda, T., et al. (2013). The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nature Communications*, *4*(1), 1–12.

Klampfer, L., Huang, J., Sasazuki, T., Shirasawa, S., & Augenlicht, L. (2003). Inhibition of Interferon γ Signaling by the Short Chain Fatty Acid Butyrate-11Montefiore Medical Center New Research Initiative Award to LK and the American Cancer Society Institutional Research Grant to LK (ACS IRG# 98-274-01), UO1 CA88104 (to LA), and P30-13330 from NCI. *Molecular Cancer Research*, *1*(11), 855–862.

Ko, Y.F., Martel, J., Wu, T.R., Chang, C.J., Lin, C.S., Liau, J.C., et al. (2018). U.S. Patent No. 10,111,915. Washington, DC: U.S. Patent and Trademark Office.

Korpela, K., Salonen, A., Hickman, B., Kunz, C., Sprenger, N., Kukkonen, K., et al. (2018). Fucosylated oligosaccharides in mother's milk alleviate the effects of caesarean birth on infant gut microbiota. *Scientific Reports*, *8*(1), 1–7.

Kothari, D., Patel, S., & Kim, S. K. (2019). Probiotic supplements might not be universally-effective and safe: A review. *Biomedicine & Pharmacotherapy*, *111*, 537–547.

Koutnikova, H., Genser, B., Monteiro-Sepulveda, M., Faurie, J. M., Rizkalla, S., Schrezenmeir, J., Clément, K. et al. (2019). Impact of bacterial probiotics on obesity, diabetes and non-alcoholic fatty liver disease related variables: A systematic review and meta-analysis of randomised controlled trials. *BMJ Open*, *9*(3), e017995.

Kuwahara, T., Yamashita, A., Hirakawa, H., Nakayama, H., Toh, H., Okada, N., et al. (2004). Genomic analysis of *Bacteroides fragilis* reveals extensive DNA inversions regulating cell surface adaptation. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(41), 14919–14924.

Lee, H., & Ko, G. (2014). Effect of metformin on metabolic improvement and gut microbiota. *Applied and Environment Microbiology*, *80*(19), 5935–5943.

Lee, Y. K., Mehrabian, P., Boyajian, S., Wu, W. L., Selicha, J., Vonderfecht, S., et al. (2018). The protective role of *Bacteroides fragilis* in a murine model of colitis-associated colorectal cancer. *mSphere*, *3*(6), e00587-18.

Li, G., Yin, B., Li, J., Wang, J., Wei, W., Bolnick, D. I., et al. (2020). Host-microbiota interaction helps to explain the bottom-up effects of climate change on a small rodent species. *The ISME Journal*, 1–14.

Li, J., Lin, S., Vanhoutte, P. M., Woo, C. W., & Xu, A. (2016). *Akkermansia muciniphila* protects against atherosclerosis by preventing metabolic endotoxemia-induced inflammation in Apoe−/− mice. *Circulation*, *133*(24), 2434–2446.

Li, K., Peng, W., Zhou, Y., Ren, Y., Zhao, J., Fu, X., et al. (2020). Host genetic and environmental factors shape the composition and function of gut microbiota in populations living at high altitude. *BioMed Research International*, *2020*, 1–10.

Li, S. Y., & Susztak, K. (2018). The role of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) in kidney disease. *Seminars in Nephrology*, *38*(2), 121–126.

Li, Z., Deng, H., Zhou, Y., Tan, Y., Wang, X., Han, Y., et al. (2017). Bioluminescence imaging to track *Bacteroides fragilis* inhibition of *Vibrio parahaemolyticus* infection in mice. *Frontiers in Cellular and Infection Microbiology*, *7*, 170.

Li, Z., Henning, S. M., Lee, R. P., Lu, Q. Y., Summanen, P. H., Thames, G., et al. (2015). Pomegranate extract induces ellagitannin metabolite formation and changes stool microbiota in healthy volunteers. *Food & Function*, *6*(8), 2487–2495.

Lopez-Siles, M., Khan, T. M., Duncan, S. H., Harmsen, H. J., Garcia-Gil, L. J., & Flint, H. J. (2012). Cultured representatives of two major phylogroups of human colonic *Faecalibacterium prausnitzii* can utilize pectin, uronic acids, and host-derived substrates for growth. *Applied and Environment Microbiology*, *78*(2), 420–428.

Lopez-Siles, M., Martinez-Medina, M., Surís-Valls, R., Aldeguer, X., Sabat-Mir, M., Duncan, S. H., et al. (2016). Changes in the abundance of *Faecalibacterium prausnitzii* phylogroups I and II in the intestinal mucosa of inflammatory bowel disease and patients with colorectal cancer. *Inflammatory Bowel Diseases*, *22*(1), 28–41.

Louis, P., & Flint, H. J. (2009). Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiology Letters*, *294*(1), 1–8.

Louis, P., Young, P., Holtrop, G., & Flint, H. J. (2010). Diversity of human colonic butyrate-producing bacteria revealed by analysis of the butyryl-CoA: Acetate CoA-transferase gene. *Environmental Microbiology*, *12*(2), 304–314.

Lukovac, S., Belzer, C., Pellis, L., Keijser, B. J., de Vos, W. M., Montijn, R. C., et al. (2014). Differential modulation by *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* of host peripheral lipid metabolism and histone acetylation in mouse gut organoids. *mBio*, *5*(4), e01438-14.

Ma, J., Sun, L., Liu, Y., Ren, H., Shen, Y., Bi, F., et al. (2020). Alter between gut bacteria and blood metabolites and the anti-tumor effects of *Faecalibacterium prausnitzii* in breast cancer. *BMC Microbiology*, *20*, 1–19.

Machiels, K., Joossens, M., Sabino, J., De Preter, V., Arijs, I., Eeckhaut, V., et al. (2014). A decrease of the butyrate-producing species *Roseburia hominis* and *Faecalibacterium prausnitzii* defines dysbiosis in patients with ulcerative colitis. *Gut*, *63*(8), 1275–1283.

Manzanares, W., Lemieux, M., Langlois, P. L., & Wischmeyer, P. E. (2016). Probiotic and synbiotic therapy in critical illness: A systematic review and meta-analysis. *Critical Care*, *20*(1), 262.

Markowiak, P., & Śliżewska, K. (2017). Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients*, *9*(9), 1021.

Martín, R., Bermúdez-Humarán, L. G., & Langella, P. (2018). Searching for the bacterial effector: The example of the multi-skilled commensal bacterium *Faecalibacterium prausnitzii*. *Frontiers in Microbiology*, *9*, 346.

Martín, R., Chain, F., Miquel, S., Lu, J., Gratadoux, J. J., Sokol, H., et al. (2014). The commensal bacterium *Faecalibacterium prausnitzii* is protective in DNBS-induced chronic moderate and severe colitis models. *Inflammatory Bowel Diseases*, *20*(3), 417–430.

Martín, R., Miquel, S., Benevides, L., Bridonneau, C., Robert, V., Hudault, S., et al. (2017). Functional characterization of novel *Faecalibacterium prausnitzii* strains isolated from healthy volunteers: A step forward in the use of *F. prausnitzii* as a next-generation probiotic. *Frontiers in Microbiology*, *8*, 1226.

Martín, R., Miquel, S., Chain, F., Natividad, J. M., Jury, J., Lu, J., et al. (2015). *Faecalibacterium prausnitzii* prevents physiological damages in a chronic low-grade inflammation murine model. *BMC Microbiology*, *15*(1), 67.

Martinez-Medina, M., Aldeguer, X., Gonzalez-Huix, F., Acero, D., & Garcia-Gil, J. L. (2006). Abnormal microbiota composition in the ileocolonic mucosa of Crohn's disease patients as revealed by polymerase chain reaction-denaturing gradient gel electrophoresis. *Inflammatory Bowel Diseases*, *12*(12), 1136–1145.

Masumoto, S., Terao, A., Yamamoto, Y., Mukai, T., Miura, T., & Shoji, T. (2016). Non-absorbable apple procyanidins prevent obesity associated with gut microbial and metabolomic changes. *Scientific Reports*, *6*, 31208.

Mazhar, S. F., Afzal, M., Almatroudi, A., Munir, S., Ashfaq, U. A., Rasool, M., et al. (2020). The prospects for the therapeutic implications of genetically engineered probiotics. *Journal of Food Quality*, *2020*, 1–11.

Mazmanian, S. K., Liu, C. H., Tzianabos, A. O., & Kasper, D. L. (2005). An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell*, *122*(1), 107–118.

Mazmanian, S. K., Round, J. L., & Kasper, D. L. (2008). A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature*, *453*(7195), 620–625.

McLellan, P., Lavelle, A., Brot, L., Straube, M., de Sordi, L., Grill, J. P., et al. (2020). P020 MAM, an anti-inflammatory protein derived from *Faecalibacterium prausnitzii* as a biomarker in Crohn's disease? *Journal of Crohn's and Colitis*, *14*(Suppl. 1), S140–S141.

Mehrabian, P., Boyajian, S., Wu, W. L., Selicha, J., Vonderfecht, S., & Mazmanian, S. K. (2018). The protective role of *Bacteroides fragilis* in a murine model of colitis-associated colorectal cancer. *mSphere*, *3*(6), e00587-18.

Miquel, S., Leclerc, M., Martin, R., Chain, F., Lenoir, M., Raguideau, S., et al. (2015). Identification of metabolic signatures linked to anti-inflammatory effects of *Faecalibacterium prausnitzii*. *mBio*, *6*(2), e00300-15.

Miquel, S., Martín, R., Lashermes, A., Gillet, M., Meleine, M., Gelot, A., et al. (2016). Anti-nociceptive effect of *Faecalibacterium prausnitzii* in non-inflammatory IBS-like models. *Scientific Reports*, *6*, 19399.

Moens, F., Weckx, S., & De Vuyst, L. (2016). Bifidobacterial inulin-type fructan degradation capacity determines cross-feeding interactions between bifidobacteria and *Faecalibacterium prausnitzii*. *International Journal of Food Microbiology*, *231*, 76–85.

Moodley, T., & Mistry, E. (2019). *Could the gut microbiome revolutionize medical care?* (pp. 1–14). Morrisville, North Carolina, United States: Syneos Health.

Munukka, E., Rintala, A., Toivonen, R., Nylund, M., Yang, B., Takanen, A., et al. (2017). *Faecalibacterium prausnitzii* treatment improves hepatic health and reduces adipose tissue inflammation in high-fat fed mice. *The ISME Journal*, *11*(7), 1667–1679.

Nagpal, R., Kumar, A., Kumar, M., Behare, P. V., Jain, S., & Yadav, H. (2012). Probiotics, their health benefits and applications for developing healthier foods: A review. *FEMS Microbiology Letters*, *334*(1), 1–15.

Natividad, J. M., & Verdu, E. F. (2013). Modulation of intestinal barrier by intestinal microbiota: Pathological and therapeutic implications. *Pharmacological Research*, *69*(1), 42–51.

Nguyen, H. T., Truong, D. H., Kouhoundé, S., Ly, S., Razafindralambo, H., & Delvigne, F. (2016). Biochemical engineering approaches for increasing viability and functionality of probiotic bacteria. *International Journal of Molecular Sciences*, *17*(6), 867.

O'Toole, P. W., Marchesi, J. R., & Hill, C. (2017). Next-generation probiotics: The spectrum from probiotics to live biotherapeutics. *Nature Microbiology*, *2*(5), 1–6.

Ochoa-Repáraz, J., Mielcarz, D. W., Ditrio, L. E., Burroughs, A. R., Begum-Haque, S., Dasgupta, S., et al. (2010). Central nervous system demyelinating disease protection by the human commensal *Bacteroides fragilis* depends on polysaccharide A expression. *The Journal of Immunology*, *185*(7), 4101–4108.

Oh, J. K., Amoranto, M. B. C., Oh, N. S., Kim, S., Lee, J. Y., Oh, Y. N., et al. (2020). Synergistic effect of *Lactobacillus gasseri* and *Cudrania tricuspidata* on the modulation of body weight and gut microbiota structure in diet-induced obese mice. *Applied Microbiology and Biotechnology*, *104*, 6273–6285.

Ohigashi, S., Sudo, K., Kobayashi, D., Takahashi, O., Takahashi, T., Asahara, T., et al. (2013). Changes of the intestinal microbiota, short chain fatty acids, and fecal pH in patients with colorectal cancer. *Digestive Diseases and Sciences*, *58*(6), 1717–1726.

Oka, A., Mishima, Y., Bongers, G., Baltus, A., Liu, B., Herzog, J., et al. (2020). P074 Human-derived VE202 strains reduce Enterobacteriaceae and Fusobacteria and reverse experimental colitis induced by human gut microbiota. *Inflammatory Bowel Diseases*, *26*(Suppl. 1), S36–S37.

Orenstein, R., Dubberke, E., Hardi, R., Ray, A., Mullane, K., Pardi, D. S., et al. (2016). Safety and durability of RBX2660 (microbiota suspension) for recurrent *Clostridium difficile* infection: Results of the PUNCH CD study. *Clinical Infectious Diseases*, *62*(5), 596–602.

Ottman, N. A. (2015). *Host immunostimulation and substrate utilization of the gut symbiont Akkermansia muciniphila*. Wageningen, the Netherlands: PhD thesis, Wageningen University.

Ottman, N., Davids, M., Suarez-Diez, M., Boeren, S., Schaap, P. J., dos Santos, V. A. M., et al. (2017). Genome-scale model and omics analysis of metabolic capacities of *Akkermansia muciniphila* reveal a preferential mucin-degrading lifestyle. *Applied and Environment Microbiology*, *83*(18), e01014-17.

Ottman, N., Geerlings, S. Y., Aalvink, S., de Vos, W. M., & Belzer, C. (2017). Action and function of *Akkermansia muciniphila* in microbiome ecology, health and disease. *Best Practice & Research Clinical Gastroenterology*, *31*(6), 637–642.

Ouwerkerk, J. P., Aalvink, S., Belzer, C., & De Vos, W. M. (2017). Preparation and preservation of viable *Akkermansia muciniphila* cells for therapeutic interventions. *Beneficial Microbes*, *8*(2), 163–169.

Ouwerkerk, J. P., van der Ark, K. C., Davids, M., Claassens, N. J., Finestra, T. R., de Vos, W. M., et al. (2016). Adaptation of *Akkermansia muciniphila* to the oxic-anoxic interface of the mucus layer. *Applied and Environment Microbiology*, *82*(23), 6983–6993.

Pagliuca, C., Cicatiello, A. G., Colicchio, R., Greco, A., Cerciello, R., Auletta, L., et al. (2016). Novel approach for evaluation of Bacteroides fragilis protective role against Bartonella henselae liver damage in immunocompromised murine model. *Frontiers in Microbiology*, *7*, 1750.

Pamer, E. G. (2016). Resurrecting the intestinal microbiota to combat antibiotic-resistant pathogens. *Science*, *352*(6285), 535–538.

Petrof, E. O., Dhaliwal, R., Manzanares, W., Johnstone, J., Cook, D., & Heyland, D. K. (2012). Probiotics in the critically ill: A systematic review of the randomized trial evidence. *Critical Care Medicine*, *40*(12), 3290–3302.

Plovier, H., Everard, A., Druart, C., Depommier, C., Van Hul, M., Geurts, L., et al. (2017). A purified membrane protein from *Akkermansia muciniphila* or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nature Medicine*, *23*(1), 107.

Png, C. W., Lindén, S. K., Gilshenan, K. S., Zoetendal, E. G., McSweeney, C. S., Sly, L. I., et al. (2010). Mucolytic bacteria with increased prevalence in IBD mucosa augment in vitro utilization of mucin by other bacteria. *American Journal of Gastroenterology*, *105*(11), 2420–2428.

Prosberg, M., Bendtsen, F., Vind, I., Petersen, A. M., & Gluud, L. L. (2016). The association between the gut microbiota and the inflammatory bowel disease activity: A systematic review and meta-analysis. *Scandinavian Journal of Gastroenterology*, *51*(12), 1407–1415.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., et al. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, *464*(7285), 59–65.

Qin, J., Li, Y., Cai, Z., Li, S., Zhu, J., Zhang, F., et al. (2012). A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*, *490*(7418), 55–60.

Quévrain, E., Maubert, M. A., Michon, C., Chain, F., Marquand, R., Tailhades, J., et al. (2016). Identification of an anti-inflammatory protein from *Faecalibacterium prausnitzii*, a commensal bacterium deficient in Crohn's disease. *Gut*, *65*(3), 415–425.

Quin, C., Estaki, M., Vollman, D. M., Barnett, J. A., Gill, S. K., & Gibson, D. L. (2018). Probiotic supplementation and associated infant gut microbiome and health: A cautionary retrospective clinical comparison. *Scientific Reports*, *8*(1), 1–16.

Rajilić-Stojanović, M., Biagi, E., Heilig, H. G., Kajander, K., Kekkonen, R. A., Tims, S., et al. (2011). Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. *Gastroenterology*, *141*(5), 1792–1801.

Remely, M., Hippe, B., Geretschlaeger, I., Stegmayer, S., Hoefinger, I., & Haslberger, A. (2015). Increased gut microbiota diversity and abundance of *Faecalibacterium prausnitzii* and *Akkermansia* after fasting: A pilot study. *Wiener klinische Wochenschrift*, *127*(9–10), 394–398.

Reunanen, J., Kainulainen, V., Huuskonen, L., Ottman, N., Belzer, C., Huhtinen, H., et al. (2015). *Akkermansia muciniphila* adheres to enterocytes and strengthens the integrity of the epithelial cell layer. *Applied and Environment Microbiology*, *81*(11), 3655–3662.

Ring, C., Klopfleisch, R., Dahlke, K., Basic, M., Bleich, A., & Blaut, M. (2019). *Akkermansia muciniphila* strain ATCC BAA-835 does not promote short-term intestinal inflammation in gnotobiotic interleukin-10-deficient mice. *Gut Microbes*, *10*(2), 188–203.

Rios-Covian, D., Gueimonde, M., Duncan, S. H., Flint, H. J., & de Los Reyes-Gavilan, C. G. (2015). Enhanced butyrate formation by cross-feeding between *Faecalibacterium prausnitzii* and *Bifidobacterium adolescentis*. *FEMS Microbiology Letters*, *362*(21), 1–7.

Rodríguez-Daza, M. C., Daoust, L., Boutkrabt, L., Pilon, G., Varin, T., Dudonné, S., et al. (2020). Wild blueberry proanthocyanidins shape distinct gut microbiota profile and influence glucose homeostasis and intestinal phenotypes in high-fat high-sucrose fed mice. *Scientific Reports*, *10*(1), 1–16.

Roopchand, D. E., Carmody, R. N., Kuhn, P., Moskal, K., Rojas-Silva, P., Turnbaugh, P. J., et al. (2015). Dietary polyphenols promote growth of the gut bacterium *Akkermansia muciniphila* and attenuate high-fat diet–induced metabolic syndrome. *Diabetes*, *64*(8), 2847–2858.

Rossi, O., Khan, M. T., Schwarzer, M., Hudcovic, T., Srutkova, D., Duncan, S. H., et al. (2015). *Faecalibacterium prausnitzii* strain HTF-F and its extracellular polymeric matrix attenuate clinical parameters in DSS-induced colitis. *PLoS One*, *10*(4), .

Rossi, O., Van Berkel, L. A., Chain, F., Khan, M. T., Taverne, N., Sokol, H., et al. (2016). *Faecalibacterium prausnitzii* A2-165 has a high capacity to induce IL-10 in human and murine dendritic cells and modulates T cell responses. *Scientific Reports*, *6*(18507), 1–12.

Round, J. L., & Mazmanian, S. K. (2010). Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(27), 12204–12209.

Routy, B., Gopalakrishnan, V., Daillère, R., Zitvogel, L., Wargo, J. A., & Kroemer, G. (2018). The gut microbiota influences anticancer immunosurveillance and general health. *Nature Reviews Clinical Oncology*, *15*(6), 382–396.

Saarela, M. H. (2019). Safety aspects of next generation probiotics. *Current Opinion in Food Science*, *30*, 8–13.

Sakamoto, M., & Benno, Y. (2006). Reclassification of *Bacteroides distasonis*, *Bacteroides goldsteinii* and *Bacteroides merdae* as *Parabacteroides distasonis* gen. nov., comb. nov., *Parabacteroides goldsteinii* comb. nov. and *Parabacteroides merdae* comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, *56*(7), 1599–1605.

Sakamoto, M., Suzuki, N., Matsunaga, N., Koshihara, K., Seki, M., Komiya, H., et al. (2009). *Parabacteroides gordonii* sp. nov., isolated from human blood cultures. *International Journal of Systematic and Evolutionary Microbiology*, *59*(11), 2843–2847.

Sanders, M. E., Akkermans, L. M., Haller, D., Hammerman, C., Heimbach, J. T., Hörmannsperger, G., et al. (2010). Safety assessment of probiotics for human use. *Gut Microbes*, *1*(3), 164–185.

Santacruz, A., Collado, M. C., Garcia-Valdes, L., Segura, M. T., Martin-Lagos, J. A., Anjos, T., et al. (2010). Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. *British Journal of Nutrition*, *104*(1), 83–92.

Sarrabayrouse, G., Alameddine, J., Altare, F., & Jotereau, F. (2015). Microbiota-specific CD4CD8αα Tregs: Role in intestinal immune homeostasis and implications for IBD. *Frontiers in Immunology*, *6*, 522.

Sbahi, H., & Di Palma, J. A. (2016). Faecal microbiota transplantation: Applications and limitations in treating gastrointestinal disorders. *BMJ Open Gastroenterology*, *3*(1), e000087.

Schneeberger, M., Everard, A., Gómez-Valadés, A. G., Matamoros, S., Ramírez, S., Delzenne, N. M., et al. (2015). *Akkermansia muciniphila* inversely correlates with the onset of inflammation, altered adipose tissue metabolism and metabolic disorders during obesity in mice. *Scientific Reports*, *5*, 16643.

Schroeder, B. O., & Bäckhed, F. (2016). Signals from the gut microbiota to distant organs in physiology and disease. *Nature Medicine*, *22*(10), 1079.

Schwab, C., Ruscheweyh, H. J., Bunesova, V., Pham, V. T., Beerenwinkel, N., & Lacroix, C. (2017). Trophic interactions of infant bifidobacteria and *Eubacterium hallii* during L-fucose and fucosyllactose degradation. *Frontiers in Microbiology*, *8*, 95.

Schwab, M., Reynders, V., Loitsch, S., Steinhilber, D., Stein, J., & Schröder, O. (2007). Involvement of different nuclear hormone receptors in butyrate-mediated inhibition of inducible NFκB signalling. *Molecular Immunology*, *44*(15), 3625–3632.

Sears, C. L. (2009). Enterotoxigenic *Bacteroides fragilis*: A rogue among symbiotes. *Clinical Microbiology Reviews*, *22*(2), 349–369.

Shen, J., Tong, X., Sud, N., Khound, R., Song, Y., Maldonado-Gomez, M. X., et al. (2016). Low-density lipoprotein receptor signaling mediates the triglyceride-lowering action of *Akkermansia muciniphila* in genetic-induced hyperlipidemia. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *36*(7), 1448–1456.

Shen, Y., Torchia, M. L. G., Lawson, G. W., Karp, C. L., Ashwell, J. D., & Mazmanian, S. K. (2012). Outer membrane vesicles of a human commensal mediate immune regulation and disease protection. *Cell Host & Microbe*, *12*(4), 509–520.

Shin, J., Noh, J. R., Chang, D. H., Kim, Y. H., Kim, M. H., Lee, E. S., et al. (2019). Elucidation of *Akkermansia muciniphila* probiotic traits driven by mucin depletion. *Frontiers in Microbiology*, *10*, 1137.

Shin, N. R., Lee, J. C., Lee, H. Y., Kim, M. S., Whon, T. W., Lee, M. S., et al. (2014). An increase in the *Akkermansia* spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. *Gut*, *63*(5), 727–735.

Shono, Y., Docampo, M. D., Peled, J. U., Perobelli, S. M., Velardi, E., Tsai, J. J., et al. (2016). Increased GVHD-related mortality with broad-spectrum antibiotic use after allogeneic hematopoietic stem cell transplantation in human patients and mice. *Science Translational Medicine*, *8*(339), 339ra71.

Sittipo, P., Lobionda, S., Choi, K., Sari, I. N., Kwon, H. Y., & Lee, Y. K. (2018). Toll-like receptor 2-mediated suppression of colorectal cancer pathogenesis by polysaccharide A from *Bacteroides fragilis*. *Frontiers in Microbiology*, *9*, 1588.

Sokol, H., Pigneur, B., Watterlot, L., Lakhdari, O., Bermúdez-Humarán, L. G., Gratadoux, J. J., et al. (2008). *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(43), 16731–16736.

Sokol, H., Seksik, P., Furet, J. P., Firmesse, O., Nion-Larmurier, I., Beaugerie, L., et al. (2009). Low counts of *Faecalibacterium prausnitzii* in colitis microbiota. *Inflammatory Bowel Diseases*, *15*(8), 1183–1189.

Sommese, L., Pagliuca, C., Avallone, B., Ippolito, R., Casamassimi, A., Costa, V., et al. (2012). Evidence of *Bacteroides fragilis* protection from *Bartonella henselae*-induced damage. *PLoS One*, *7*(11), 1–13.

Song, D., Ibrahim, S., & Hayek, S. (2012). Recent application of probiotics in food and agricultural science. *Probiotics*, *10*, 1–34.

Sonoyama, K., Fujiwara, R., Takemura, N., Ogasawara, T., Watanabe, J., Ito, H., et al. (2009). Response of gut microbiota to fasting and hibernation in Syrian hamsters. *Applied and Environment Microbiology*, *75*(20), 6451–6456.

Suez, J., Zmora, N., & Elinav, E. (2020). Probiotics in the next-generation sequencing era. *Gut Microbes*, *11*(1), 77–93.

Suez, J., Zmora, N., Segal, E., & Elinav, E. (2019). The pros, cons, and many unknowns of probiotics. *Nature Medicine*, *25*(5), 716–729.

Sun, F., Zhang, Q., Zhao, J., Zhang, H., Zhai, Q., & Chen, W. (2019). A potential species of next-generation probiotics? The dark and light sides of *Bacteroides fragilis* in health. *Food Research International*, *126*, 108590.

Swidsinski, A., Loening-Baucke, V., Vaneechoutte, M., & Doerffel, Y. (2008). Active Crohn's disease and ulcerative colitis can be specifically diagnosed and monitored based on the biostructure of the fecal flora. *Inflammatory Bowel Diseases*, *14*(2), 147–161.

Takahashi, K., Nishida, A., Fujimoto, T., Fujii, M., Shioya, M., Imaeda, H., et al. (2016). Reduced abundance of butyrate-producing bacteria species in the fecal microbial community in Crohn's disease. *Digestion*, *93*(1), 59–65.

Terpou, A., Papadaki, A., Lappa, I. K., Kachrimanidou, V., Bosnea, L. A., & Kopsahelis, N. (2019). Probiotics in food systems: Significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. *Nutrients*, *11*(7), 1591.

Tsai, Y. L., Lin, T. L., Chang, C. J., Wu, T. R., Lai, W. F., Lu, C. C., et al. (2019). Probiotics, prebiotics and amelioration of diseases. *Journal of Biomedical Science*, *26*(1), 1–8.

Udayappan, S., Manneras-Holm, L., Chaplin-Scott, A., Belzer, C., Herrema, H., Dallinga-Thie, G. M., et al. (2016). Oral treatment with *Eubacterium hallii* improves insulin sensitivity in db/db mice. *NPJ Biofilms and Microbiomes*, *2*(1), 1–10.

Vaishnava, S., Yamamoto, M., Severson, K. M., Ruhn, K. A., Yu, X., Koren, O., et al. (2011). The antibacterial lectin RegIIIγ promotes the spatial segregation of microbiota and host in the intestine. *Science*, *334*(6053), 255–258.

van de Wouw, M., Walsh, A. M., Crispie, F., van Leuven, L., Lyte, J. M., Boehme, M., et al. (2020). Distinct actions of the fermented beverage kefir on host behaviour, immunity and microbiome gut-brain modules in the mouse. *Microbiome*, *8*, 1–20.

van Passel, M. W., Kant, R., Zoetendal, E. G., Plugge, C. M., Derrien, M., Malfatti, S. A., et al. (2011). The genome of *Akkermansia muciniphila*, a dedicated intestinal mucin degrader, and its use in exploring intestinal metagenomes. *PLoS One*, *6*(3), 1–8.

van Tongeren, S. P., Slaets, J. P., Harmsen, H. J. M., & Welling, G. W. (2005). Fecal microbiota composition and frailty. *Applied and Environment Microbiology*, *71*(10), 6438–6442.

Varela, E., Manichanh, C., Gallart, M., Torrejón, A., Borruel, N., Casellas, F., et al. (2013). Colonisation by *Faecalibacterium prausnitzii* and maintenance of clinical remission in patients with ulcerative colitis. *Alimentary Pharmacology & Therapeutics*, *38*(2), 151–161.

Venugopalan, V., Shriner, K. A., & Wong-Beringer, A. (2010). Regulatory oversight and safety of probiotic use. *Emerging Infectious Diseases*, *16*(11), 1661.

Vermeiren, J., Van den Abbeele, P., Laukens, D., Vigsnæs, L. K., De Vos, M., Boon, N., et al. (2012). Decreased colonization of fecal *Clostridium coccoides/Eubacterium rectale* species from ulcerative colitis patients in an in vitro dynamic gut model with mucin environment. *FEMS Microbiology Ecology*, *79*(3), 685–696.

Vétizou, M., Pitt, J. M., Daillère, R., Lepage, P., Waldschmitt, N., Flament, C., et al. (2015). Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science*, *350*(6264), 1079–1084.

Vigsnæs, L. K., Brynskov, J., Steenholdt, C., Wilcks, A., & Licht, T. R. (2012). Gram-negative bacteria account for main differences between faecal microbiota from patients with ulcerative colitis and healthy controls. *Beneficial Microbes*, *3*(4), 287–297.

Wang, B., Yao, M., Lv, L., Ling, Z., & Li, L. (2017). The human microbiota in health and disease. *Engineering*, *3*(1), 71–82.

Wang, H., Jatmiko, Y. D., Bastian, S. E., Mashtoub, S., & Howarth, G. S. (2017). Effects of supernatants from *Escherichia coli* Nissle 1917 and *Faecalibacterium prausnitzii* on intestinal epithelial cells and a rat model of 5-fluorouracil-induced mucositis. *Nutrition and Cancer*, *69*(2), 307–318.

Wang, L., Tang, L., Feng, Y., Zhao, S., Han, M., Zhang, C., et al. (2020). A purified membrane protein from *Akkermansia muciniphila* or the pasteurised bacterium blunts colitis associated tumourigenesis by modulation of CD8+ T cells in mice. *Gut*, *69*, 1988–1997.

Wang, Y., Deng, H., Li, Z., Tan, Y., Han, Y., Wang, X., et al. (2017). Safety evaluation of a novel strain of *Bacteroides fragilis*. *Frontiers in Microbiology*, *8*, 435.

Wei, Z., Cao, S., Liu, S., Yao, Z., Sun, T., Li, Y., et al. (2016). Could gut microbiota serve as prognostic biomarker associated with colorectal cancer patients' survival? A pilot study on relevant mechanism. *Oncotarget*, *7*(29), 46158.

Wen, L., & Duffy, A. (2017). Factors influencing the gut microbiota, inflammation, and type 2 diabetes. *The Journal of Nutrition*, *147*(7), 1468S–1475S.

Wexler, A. G., & Goodman, A. L. (2017). An insider's perspective: Bacteroides as a window into the microbiome. *Nature Microbiology*, *2*(5), 1–11.

Willing, B., Halfvarson, J., Dicksved, J., Rosenquist, M., Järnerot, G., Engstrand, L., et al. (2009). Twin studies reveal specific imbalances in the mucosa-associated microbiota of patients with ileal Crohn's disease. *Inflammatory Bowel Diseases*, *15*(5), 653–660.

Wrigley, D. M. (2004). Inhibition of *Clostridium perfringens* sporulation by *Bacteroides fragilis* and short-chain fatty acids. *Anaerobe*, *10*(5), 295–300.

Wu, H., Esteve, E., Tremaroli, V., Khan, M. T., Caesar, R., Mannerås-Holm, L., et al. (2017). Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. *Nature Medicine*, *23*(7), 850.

Wu, P.I. (2020). U.S. Patent Application No. 16/541,308.

Wu, P.I., Chang, C.J., & Tsai, Y.L. (2020). U.S. Patent Application No. 16/558,766.

Wu, P.I., Chang, C.J., Tsai, Y.L., & Lin, T.L. (2020). U.S. Patent Application No. 16/558,379.

Wu, T. R., Lin, C. S., Chang, C. J., Lin, T. L., Martel, J., Ko, Y. F., et al. (2019). Gut commensal *Parabacteroides goldsteinii* plays a predominant role in the anti-obesity effects of polysaccharides isolated from *Hirsutella sinensis*. *Gut*, *68*(2), 248–262.

Wu, W., Lv, L., Shi, D., Ye, J., Fang, D., Guo, F., et al. (2017). Protective effect of *Akkermansia muciniphila* against immune-mediated liver injury in a mouse model. *Frontiers in Microbiology*, *8*, 1804.

Xu, J., Liang, R., Zhang, W., Tian, K., Li, J., Chen, X., et al. (2020). *Faecalibacterium prausnitzii*-derived microbial anti-inflammatory molecule regulates intestinal integrity in diabetes mellitus mice via modulating tight junction protein expression. *Journal of Diabetes*, *12*(3), 224–236.

Xu, Y., Wang, N., Tan, H. Y., Li, S., Zhang, C., & Feng, Y. (2020). Function of *Akkermansia muciniphila* in obesity: Interactions with lipid metabolism, immune response and gut systems. *Frontiers in Microbiology*, *11*, 219.

Yang, Y., Zhong, Z., Wang, B., Xia, X., Yao, W., Huang, L., et al. (2019). Early-life high-fat diet-induced obesity programs hippocampal development and cognitive functions via regulation of gut commensal *Akkermansia muciniphila*. *Neuropsychopharmacology*, *44*(12), 2054–2064.

Yao, M., Xie, J., Du, H., McClements, D. J., Xiao, H., & Li, L. (2020). Progress in microencapsulation of probiotics: A review. *Comprehensive Reviews in Food Science and Food Safety*, *19*(2), 857–874.

Yassour, M., Lim, M. Y., Yun, H. S., Tickle, T. L., Sung, J., Song, Y. M., et al. (2016). Sub-clinical detection of gut microbial biomarkers of obesity and type 2 diabetes. *Genome Medicine*, *8*(1), 17.

Yu, S., Xiong, Y., Xu, J., Liang, X., Fu, Y., Liu, D., et al. (2020). Identification of dysfunctional gut microbiota through rectal swab in patients with different severity of acute pancreatitis. *Digestive Diseases and Sciences*, *65*, 1–15.

Zackular, J. P., Baxter, N. T., Iverson, K. D., Sadler, W. D., Petrosino, J. F., Chen, G. Y., et al. (2013). The gut microbiome modulates colon tumorigenesis. *mBio*, *4*(6), e00692-13.

Zella, G. C., Hait, E. J., Glavan, T., Gevers, D., Ward, D. V., Kitts, C. L., et al. (2011). Distinct microbiome in pouchitis compared to healthy pouches in ulcerative colitis and familial adenomatous polyposis. *Inflammatory Bowel Diseases*, *17*(5), 1092–1100.

Zhai, Q., Feng, S., Arjan, N., & Chen, W. (2019). A next generation probiotic, *Akkermansia muciniphila*. *Critical Reviews in Food Science and Nutrition*, *59*(19), 3227–3236.

Zhang, L. I., Carmody, R. N., Kalariya, H. M., Duran, R. M., Moskal, K., Poulev, A., et al. (2018). Grape proanthocyanidin-induced intestinal bloom of *Akkermansia muciniphila* is dependent on its baseline abundance and precedes activation of host genes related to metabolic health. *The Journal of Nutritional Biochemistry*, *56*, 142–151.

Zhang, L., Qin, Q., Liu, M., Zhang, X., He, F., & Wang, G. (2018). *Akkermansia muciniphila* can reduce the damage of gluco/lipotoxicity, oxidative stress and inflammation, and normalize intestine microbiota in streptozotocin-induced diabetic rats. *Pathogens and Disease*, *76*(4), fty028.

Zhang, M., Qiu, X., Zhang, H., Yang, X., Hong, N., Yang, Y., et al. (2014). *Faecalibacterium prausnitzii* inhibits interleukin-17 to ameliorate colorectal colitis in rats. *PLoS One*, *9*(10), 1–10.

Zhang, P., Li, L., Bao, Z., & Huang, F. (2016). Role of BAF60a/BAF60c in chromatin remodeling and hepatic lipid metabolism. *Nutrition & Metabolism*, *13*(1), 30.

Zhang, T., Li, Q., Cheng, L., Buch, H., & Zhang, F. (2019). *Akkermansia muciniphila* is a promising probiotic. *Microbial Biotechnology*, *12*(6), 1109–1125.

Zhang, W., Zhu, B., Xu, J., Liu, Y., Qiu, E., Li, Z., et al. (2018). *Bacteroides fragilis* protects against antibiotic-associated diarrhea in rats by modulating intestinal defenses. *Frontiers in Immunology*, *9*, 1040.

Zhang, X., Li, L., Butcher, J., Stintzi, A., & Figeys, D. (2019). Advancing functional and translational microbiome research using meta-omics approaches. *Microbiome*, *7*(1), 154.

Zhang, X., Shen, D., Fang, Z., Jie, Z., Qiu, X., Zhang, C., et al. (2013). Human gut microbiota changes reveal the progression of glucose intolerance. *PLoS One*, *8*(8), 1–11.

Zhao, S., Liu, W., Wang, J., Shi, J., Sun, Y., Wang, W., et al. (2017). *Akkermansia muciniphila* improves metabolic profiles by reducing inflammation in chow diet-fed mice. *Journal of Molecular Endocrinology*, *58*(1), 1–14.

Zhao, Y., Chen, F., Wu, W., Sun, M., Bilotta, A. J., Yao, S., et al. (2018). GPR43 mediates microbiota metabolite SCFA regulation of antimicrobial peptide expression in intestinal epithelial cells via activation of mTOR and STAT3. *Mucosal Immunology*, *11*(3), 752–762.

Zhou, B., Xia, X., Wang, P., Chen, S., Yu, C., Huang, R., et al. (2018). Induction and amelioration of methotrexate-induced gastrointestinal toxicity are related to immune response and gut microbiota. *EBioMedicine*, *33*, 122–133.

Zmora, N., Suez, J., & Elinav, E. (2019). You are what you eat: Diet, health and the gut microbiota. *Nature Reviews Gastroenterology & Hepatology*, *16*(1), 35–56.

Zmora, N., Zilberman-Schapira, G., Suez, J., Mor, U., Dori-Bachash, M., Bashiardes, S., et al. (2018). Personalized gut mucosal colonization resistance to empiric probiotics is associated with unique host and microbiome features. *Cell*, *174*(6), 1388–1405.

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Chapter 5

Edible Mushrooms: A Promising Bioresource for Prebiotics

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1 Introduction

Mushrooms are defined as macrofungi that have been valued as both food and traditional medicine throughout the world for thousands of years. It may grow above and below the ground. They have a reproductive structure that means fruiting bodies. At the bottom of the fruiting bodies, gills are attached. This gill carries a tiny spore and thus helps one to germinate and grow a new one. A new individual started to grow a long, thin filaments called hyphae. The body of most fungi is made up of an immense bunch of hyphae, all loops interconnected to form a convoluted network called mycelium. When mycelia have grown sufficiently forming fruiting bodies, such mushrooms can be produced (Loria-Kohen et al., 2014).

There are lots of fungi are available. Of all fungi, nearly 12,000 species are considered as mushrooms. Some of 2000 species are used for edible, 200 species were collected as wild and are used for traditional purposes. Commercial species are cultivated about 35 species and for industrial scale are about 20 species (Aida et al., 2009).

In recent years, the intake of mushrooms has been raised. Mushrooms are rich in bioactive metabolites, including polysaccharides, proteins (ribosome inactivating proteins), dietary fibers (β-glucans), enzymes (e.g., ribonucleases, superoxide dismutase, and laccases), and many other biomolecules (lectins, antifungal proteins, secondary metabolites, protease inhibitors, and ubiquitin-like proteins) that act as a significant nutritional supplement. Different bioactive compounds have been isolated from different kinds of mushrooms. The bioactive compounds provide beneficial properties such as antimicrobial, antioxidant, immunomodulatory, cardiovascular, antiinflammatory, and antidiabetic (Guillamon et al., 2010; Kosanic et al., 2012; Elsayed et al., 2014; Ganeshpurkar et al., 2014; Guggenheim et al., 2014). Some research has been analyzing the mushroom's active composition for many functions as diet food (Akindahunsi & Oyetayo, 2006; Rao et al., 2009) and pharmaceutical (Siqian & Shah, 2015; Valverde, Hernández-pérez, & Paredes-lópez, 2015) and cosmeceutical (Taofiq et al., 2016) applications (Sawangwan et al., 2018). The prevention and treatment of several human health hazards is successful.

Mushroom quality is evaluated by the combination of parameters that include texture, development stage, whiteness, and microbial counts. The most important parameter is the color change (Fig. 5.1).

Diet composition and dietary additives such as prebiotics, antibiotics influencing the intestinal environment and thus may alter the composition and action of animal microbiota (Guo et al., 2003). Food containing prebiotics are commonly consumed by humans.

Gibson and Roberfroid (1995) introduced the term prebiotic, and they convert "pro" to "pre," which means "before" or "for" (Schrezenmeir & Michael, 2001; Aida et al., 2009). Prebiotics are fermented food ingredients or nondigestive components of a diet that induce the host by their selective growth or activation of specific microorganisms present in intestines

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00005-8
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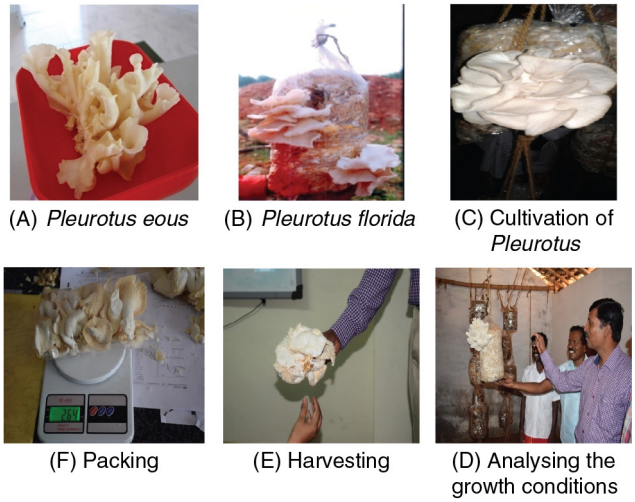


FIGURE 5.1 Cultivation of edible *Pleurotus* spp. (A) *Pleurotus eous*, (B) *Pleurotus florida*, (C) cultivation of *Pleurotus*, (D) analyzing the growth conditions, (E) harvesting, and (F) packing.

(Toghyani et al., 2012). It is the primary energy source in gut microflora (Khangwal & Shukla, 2019). The prebiotics are the fragments of carbohydrates, including oligosaccharides of galactose, fructose or mannose, and polysaccharides, and are regarded as functional foods (Singdevsachan et al., 2016). By enzymatic digestions, fruits and vegetables occur naturally or can be made synthetically.

All dietary carbohydrates are not prebiotics, but some oligosaccharides and polysaccharides (including dietary fibers) take into prebiotic activity (Gibson, 2004; Gibson et al., 2004). Prebiotics food ingredients are not intent or hydrolyzed in the upper gastrointestinal tract (GIT). It should be a selective substrate for a limited number of colonic bacteria, which influences the luminal or systematic effects and alters the healthier composition that is favorable to the host health in the colon (Ziemer & Gibson, 1998).

Prebiotics play an individual role in human's nutrition. It fully focuses on manipulation bacterial activities that colonize our bodies (Douglas & Sanders, 2008). Consumption of prebiotic includes enhanced immune function, reduces long-chain fatty acids (LCFAs) in bowels, enhances immune function, improves colonic integrity, lessens the intestinal infections duration, downregulates allergic response for a betterment of digestion and elimination (Douglas & Sanders, 2008). Beneficial intestinal bacteria metabolize these responses. The prebiotic substrates are available in some beneficial bacteria such as bifidogenic bacteria (*Bifidobacterium* sp.), lactic acid bacteria (*Lactobacillus* sp., *Leuconostoc*, *Pediococcus*, *Lactococcus*, *Streptococcus*), and lesser to pathogenic bacteria such as toxin producing *Clostridium* sp., proteolytic *Bacteroides*, and toxigenic *Escherichia coli* (Samal & Behura, 2018). Some of these bacteria are beneficial to the host by reducing the intestinal pathogens and/or modifying the production of health-related bacterial metabolites (Ringo et al., 2010).

Prebiotic compounds are one of the health-promoting compounds which are developed as a dietary supplementation strategy compared with antibiotics (Rahman et al., 2012). Mostly plant-derived additives and dietary fibers are prebiotic compounds, which have an interesting application in aquaculture by suppressing the deleterious bacteria and stimulating the health of gut.

During the past years, fibers are considered as prebiotics, but the recent studies indicated that prebiotics show a behavior of fiber compounds. The usage of prebiotics is an optimistic advance technique for improving the function of endogenic beneficial microbiota in the intestine. It can also be used as a possible alternative to growth promoting antibiotics (Ringo & Olsen, 1999; Ganguly et al., 2010). All the fibers do not have prebiotic effects because some prebiotics do not contain fiber. In addition, fiber increases the lactic fermentation because prebiotics favor the growth of lactic acid bacteria (Mallik, 2018). For maintaining a good health of an individual, gut microflora is responsible and the prebiotic consumption influences the microbes directly (Khangwal & Shukla, 2019).

Recently, the development of prebiotic is aimed at mushroom that contains carbohydrates, acts as a potential prebiotic, and also has been connected with various promoting effects of health (Rahman et al., 2012). Mushrooms act as a source of a potential prebiotics substrate in human gut microbiota. The presence of high nondigestible materials in mushroom may encourage the growth of probiotics in the GIT. The prebiotic effect of mushrooms promotes health (Solano-Aguilar et al., 2018). Mushroom supplementations as a prebiotic and its effects on growth performance were studied. Mushroom



FIGURE 5.2 *Ganoderma lucidum.*

polysaccharides contain prebiotic effects, and some of the crude polysaccharide mushrooms are *Flammulina velutipes*, *Ganoderma lucidum* (Fig. 5.2), *Pleurotus eryngii*, and *Lentinus edodes* (Yamin et al., 2012).

Prebiotic is not consumed directly, and the strategies to overcome these are encapsulation, entrapment, and immobilization (Cui et al., 2018). The study finds out prebiotics with some new natural sources. The intake of prebiotics may additionally need numerous positive fitness benefits, progress colonic integrity, immune characteristics, decrease allergic reaction, and advance digestion and excretion.

Currently, various foods are used as prebiotics. Mushrooms can also serve as a good prebiotic because of the rich sources of carbohydrates (Chandrasekaran et al., 2011).

1.1 Mushrooms as food values

Nowadays, food is not barely taken to mollify hunger. It supplies the required essential nutrients to the customers from diseases and improves health. The foods that essentially and beneficially act to human are known as functional foods. According to the Institute of Medicine's Food and Nutrition Board, "Functional Foods" dietary part of food not only provide nutrition but also give health benefits (Thatoi et al., 2018). Foods containing proteins, minerals, and vitamins are necessary in addition to that furnishing energy. Protein-rich foods are important in human health. Though protein is synthesized tremendously by green plants, the concentration of protein plants with few exceptions is quite low in terms of the percentage of total weight. Mushroom's nutritional value lies between meat and vegetables. Mushrooms are considered as flush where they have a higher functional and nutritional value. A high nutritional value of mushrooms is found in the form of high content of protein (up to 44.93%), low calories, carbohydrates, valuable salts, fibers, trace elements, which lack cholesterol. Mushroom contains protein that consists of various amino acids. All the essential amino acids are present in mushrooms. Due to its bioactive substances, mushrooms have been established as dietary supplements to treat and prevent many disorders (Rathore et al., 2017). The use of natural immunostimulants such as mushrooms is a successful growth and is able to solve problems for the usage of antibiotics. On the other hand, while investigating, the mushroom's chemical structure, or its extracts and extraction protocols of biological activities are considered extracts. These water extracts trigger the resistant cells while the ethanol extracts suppress them (Martel, 2017) (Table 5.1).

1.2 Energy value of mushrooms

Mushrooms are a good source of energy in the form of proteins, which contain 90% water by weight. The remaining 10% consists of 10%–40% protein, 2%–8% fat, 3%–28% carbohydrate, 3%–32% fiber, and 8%–10% mineral components (Borchers et al., 1999). It provides low calories and a low amount of fat, some main fatty acids such as linoleic, oleic, and palmitic. Usually edible mushrooms contain higher contents of ash, including magnesium, potassium, calcium, iron, phosphorous, zinc, and copper, and these dry matters are about 80–120 g/kg. High proportions of carbohydrates come in the form of chitin, mannitol, trehalose, and glycogen. Fibers, pectic substances, β-glucans, and hemicellulose are also in mushrooms. Sugars in the form of glucose with huge amount, fructose and sucrose with low amount were additionally present (Mattila et al., 2001, 2002). These also contain higher level of vitamins B1, B2 (riboflavin), B12, C, and D, folates, and niacin (Valverde, Hernández-pérez, & Paredes-lópez, 2015). The advantage of the mushrooms is that it has the capacity of converting high protein food from valueless substances.

In India, where vegetarians predominate, every attempt should be made to popularize a vegetable protein source like mushroom. Mushrooms are the only nonanimal food containing vitamin D. It is a good source of food for vegetarians.

TABLE 5.1 Worldwide production of mushroom.

Country	Production
Africa	28,767
Australia	51,222[a]
Canada	138,412
China	6,675,364[a]
Europe	1,324,198[a]
India	60,733[b]
Indonesia	31,052
Iran	81,406[b]
Italy	70,673
Japan	65,747[b]
Jordan	935[b]
Kazakhstan	510[c]
The Netherlands	300,000
Republic of Korea	22,737[b]
Singapore	108
Thailand	1198[b]
The United Kingdom	98,500
The United States of America	416,050
Viet Nam	23,659[b]
World	8,993,280[a]

[a]*Calculated data.*
[b]*FAO data based on imputation methodology.*
[c]*FAO estimate.*
Source:(FAO, 2020).

Mushroom acts as a promising prebiotic that has several health-promoting effects. Prebiotics are food components fulfilling particularly beyond the nutrition.

1.3 Mushrooms in India

In Asian countries, mushroom cultivation had started centuries ago. Commercial mushroom growing was first initiated in New Delhi and Solan, and later it spread to Jammu & Kashmir, Tamil Nadu (the Nilgiris), Punjab, Haryana, Chandigarh, Uttar Pradesh, Maharashtra, Madhya Pradesh, and Gujarat.

1.4 Bioactive compounds of mushrooms

In the 20th century, people all over the world widely consumed mushrooms. Mushrooms contain biologically active compounds known to be bioactive compounds (Wasser, 2002). It produces secondary metabolite with a composite of biological actions and acts as an important biosource. It plays a new role in innovative medicine. Particularly, mushrooms contain an indeterminate source of biological complexes with innumerable varieties of medicinal properties. It includes low-molecular-weight and high-molecular-weight compounds (Fig. 5.3) (Thatoi et al., 2018).

1.5 Low-molecular and high-molecular weight compounds in mushroom

Mushrooms have bioactive complexes with low-molecular and high-molecular weight compounds. Low-molecular-weight compounds are triacylglycerols, quinones, cerebrosides, isoflavones, catechols, amines, sesquiterpenes, organic germanium, steroids and selenium, whereas high-molecular-weight compounds are proteins, proteoglycans, glycoproteins, glycopeptides, homo and heteropolysaccharides, glycans, and RNA-protein complexes (Fig. 5.4).

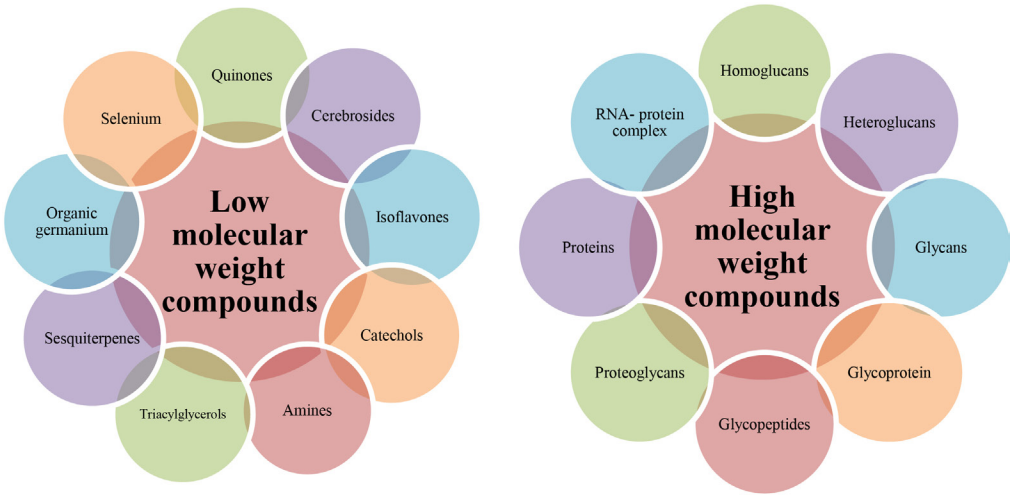


FIGURE 5.3 Bioactive compounds of mushrooms.

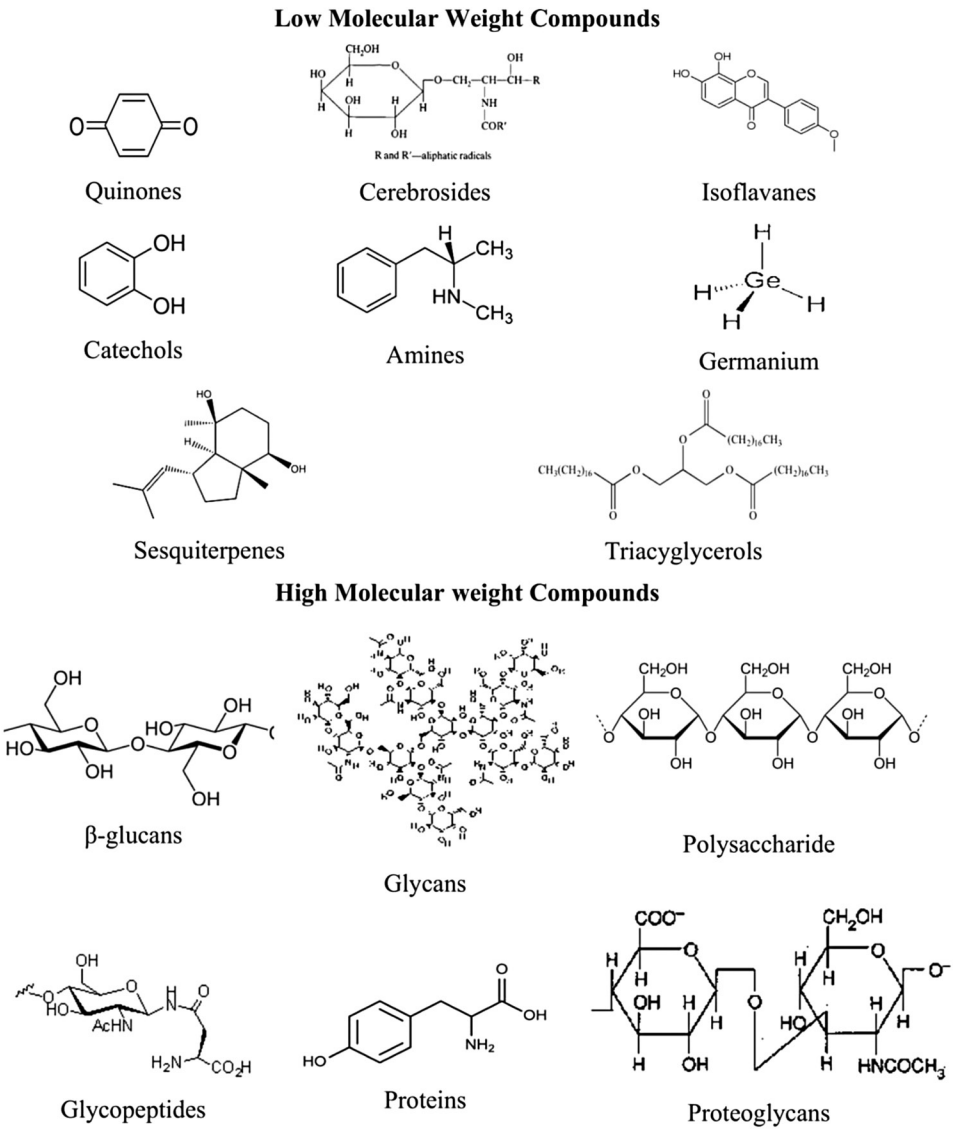


FIGURE 5.4 Chemical structure of low molecular weight and high molecular compounds in mushroom.

Mushrooms containing secondary metabolites are complex organic compound, and these compounds are used to treat many health problems, including cancer, microbial infections, and free radical related diseases (Zaidman et al., 2005). For example, *Agrocybe aegerita*, an edible mushroom, has radical scavenging and anticancer activity.

In medical mushrooms, high-molecular-weight compounds have been isolated. The lentinan is procured from the fruiting bodies of *L. edodes*, "Schizophyllan." Polysaccharides and β-glucans compounds obtain immunomodulating properties (Zhang et al., 2007).

1.6 Importance of prebiotics

Prebiotics act as a prime energy root for gut microflora (Khangwal & Shukla, 2019). The usual prebiotics consumption includes improving functions of immune, enhancing colonic integrity, lowering the intestinal infections and its duration, downregulating allergic response, and improving the digestion and elimination (Douglas & Sanders, 2008). Prebiotics may have other positive effects. Prebiotic fibers are a class of various carbohydrate ingredients. The importance of prebiotics concerns that

1. it is strongly believed that prebiotic is for healthy and balanced gut microbiota;
2. it alters the microbiota composition to serve healthier life;
3. it alters probiotics in some foodstuffs which help health benefits in the prevention of diarrhea and immunomodulation;
4. it generally helps inulin and its derivatives to be similar to plant extracts and favorable to gut microbiota and host; and
5. its functional foods give fat based and dairy products that provide organoleptic properties.

1.7 Prebiotic concept

Prebiotics are components of nondigestible diet that contributes the growth and health of the host. The prebiotics are metabolized by specific microorganisms. The prebiotic concept is carried out by ideal criteria as mentioned by Gibson et al. (2004), namely, gastric acidity resistance, gastrointestinal absorption, and mammalian enzymes that will be hydrolyzed; selective stimulation and intestinal microflora growth that are fermented; intestinal microbe activity that is associated with host well-being and health. Mushroom polysaccharide undergoes gastric juice artificially in human by the digestibility in the stomach (Musatto & Mancilha, 2007; Nowak et al., 2018). Prebiotics have some main concepts. It should

1. exist in the stomach in acidic conditions;
2. elude in small intestine;
3. selectively ferment in the colon; and
4. stimulate one or infinite counts of beneficial bacteria within the colonic microbiota, usually bifidobacterial.

1.8 Prebiotic index

Prebiotic index (PI) is defined as a measure quantity of the fermentation selectivity. It describes the correlation between the accumulation of beneficial bacteria like *Bifidobacteria* and *Lactobacilli* and correlates the variation of total count of bacteria such as clostridia and *Bacteroides* that are undesirable bacteria (Chaikliang et al., 2015). Gibson and Roberfroid defined the following:

> *A prebiotic is a nondigestible ingredient that specifically changes the gastrointestinal microflora's constitution and action in the host that gives benefits to health.*

These authors revised and found the equalization of "prebiotic" and "bifidogenic," which are defined and known as *prebiotic index*. This PI gives a rise of the fecal bifidobacterial concentration of consumed probiotics per day. Moreover, the effect of prebiotic is measured as PI (PI = *E*/*A*), which is well defined as the growth of *bifidiobacteria* expressed as a total count of feces (*E*) divided by the ingested prebiotic dose (A) (Lam & Cheung, 2013; Roberfroid, 2007).

Alterations at the level of beneficial organisms from initial population are generally measured as PI. PI is the measurement of prebiotic effects in vitro. *Bifidobacteria* and *Lactobacilli*'s populations are calculated as positive effect, while reductions in *Bacteroides* and clostridia are negative. The growth of *Bifidobacteria* in mushrooms was measured for PI. The following equation is used to calculate the PI:

$$\text{Prebiotic index} = \frac{\text{Bif}}{\text{Total}} - \frac{\text{Bac}}{\text{Total}} + \frac{\text{Lac}}{\text{Total}} - \frac{\text{Clos}}{\text{Total}}$$

where Bif is bifidobacteria numbers at sample time/numbers at inoculation; Bac is *Bacteroides* numbers at sample time/numbers at inoculation; Lac is *lactobacilli* numbers at sample time/numbers at inoculation; Clos is Clostridia numbers at sample time/numbers at inoculation; and Total is total bacteria numbers at sample time/numbers at inoculation.

TABLE 5.2 Prebiotics derived from various sources (Singla & Chakkaravarthi, 2017).

S. no.	Prebiotics derived from various sources	
1.	Plants	Beans, cabbage, onion, brussels, radish, sprouts, broccoli, asparagus, garlic, other vegetables, fruits such as apple, banana, kiwi, cereals and whole grains oats, barley
2.	Mammals	Cow's milk Human milk (Bode, 2009; Barile & Rastall, 2013)
3.	Microbes	*Trichoderma* spp., *Humicola* spp., *Bacillus pumilus, Aspergillus oryzae, Cryptococcus laurentii*
4.	Mushrooms	*Agaricus bisporus, Auricularia judae, Boletus erythropus, Ganoderma lucidum, Hericium erinaceus, Pleurotus eryngii, Pleurotus ostreatus, Lentinus edodes, Flammulina velutipes, Tremella fuciformis*
5.	Seaweeds	*Ascophyllum nodosum, Chondrus crispus, Enteromorpha prolifera, Sarcodiotheca gaudichaudii, Saccorhiza polyschides, Laminaria japonica, Laminaria digitata, Ecklonia radiate, Sargassum muticum, Kappaphycus alvarezii, Osmundea pinnatifida, Gracilaria rubra* (Kong et al., 2016; Huebbe et al., 2017)

Here, some of the criteria that analyze the PI are (1) prebiotic daily dose, (2) prebiotic nature, (3) volunteers count, and (4) *Bifidobacteria* number at the beginning and end of period at supplementation of prebiotics (Roberfroid, 2007).

1.9 Benefits of prebiotics

The health benefits of prebiotics prevent diarrhea or constipation caused by pathogens, metabolism of the intestinal flora modulation, prevention of cancer, lipid metabolism's positive effects, mineral adsorption stimulant, and immunomodulatory properties. Prebiotics emerge from different sources from plants, mammals, and some microbes. These are listed in Table 5.2.

Prebiotics act as a better treatment for several infections and diseases for both in clinical and animal studies. Molecular mechanisms of prebiotics follow the effects directly and indirectly on the gut resistant. This will help for the treatment of diseases and better therapy.

1. Prebiotics improve the beneficial bacterial growth and decrease the overgrowth of pathogenic bacteria within the gut.
2. It slows the absorption of glucose and decreases insulin that efficaciously enhances the glucose level in blood.
3. It promotes lipid profile, reduces cholesterol and triglycerides even as elevating cholesterol; additionally, it lowers the hazards of coronary cardiovascular diseases.
4. It prevents the infection of the leaky gut syndrome and intestinal lining and gives relief from continual irritation.
5. It helps the immune device to adapt to and prevent from infections and autoimmune problems such as hypersensitive reactions, bronchial asthma, and extreme diseases.
6. It supports the fermentation in intestine from soluble fiber to short-chain fatty acids (SCFAs) and offers vitamins to the mucosal lining of the gut.
7. They are favorable to Crohn's ailment via the manufacturing of quick-chain fatty acids to sustain the colon partitions and useful to ulcerative colitis via the degradation of hydrogen sulfide fuel because of the discount of sulfate-generating bacteria.
8. It has the capacity outcomes on calcium and other mineral absorption, bowel pH, discount of colorectal cancer hazard, inflammatory bowel disorder, and intestinal irregularity.

1.10 Properties of prebiotics

The prebiotics have some ideal properties that are active at low dosage, (2) regulate gut viscosity, (3) have low calorific values, (4) have ability to control microflora modulation, (5) maintain the incorporation of the feed or ration easily, (6) reduce harmful microbial loads, (7) stimulate beneficial gut microbes, (8) reduce side effects, (9) are effective at low concentration, (10) maintain acceptable storage and processing stability, (11) are noncarcinogenic, (12) produce no residual

effects, (13) maintain persistence through the colon, (14) derive from dietary polysaccharides, (15) help reduction of gas production, (16) keep varying sweetness, (17) enable to produce high selective fermentation, (18) keep rising persistence through the colon, (19) support attenuation of virulence of pathogens, and (20) enhance the existence property of antiadhesive properties against harmful gut microbes (Pandey et al., 2015; Ganguly et al., 2010).

1.11 Characteristics of ideal prebiotics

The characteristics of ideal prebiotics are (1) mammalian enzymes or tissues hydrolyzing prebiotics, (2) enriching a limited number of beneficial bacteria, (3) enhancing action of microbiota in intestine change, and (4) changing beneficially the luminal or systemic extent of the host defense system.

1.12 Mechanism of prebiotics

Prebiotics are undigested directly by the host metabolic enzymes. In GITs, the microbes digest the substrates and support the beneficial microorganism count with the help of prebiotics. So, the organism is indirectly affected by prebiotics. Prebiotic molecular structure is essential to consider the physiological effects. It determines the prebiotic action against microbes which are actually capable of utilizing. The activity of prebiotic in the microorganism influences the growth and the number in a large bowel (Monali & Kumar, 2018).

Potential mechanism of the food residues affects the gut microbiota on the mucosal layer in the gut lumen, demonstrated in Fig. 5.5. Addition of the diet-like mushrooms gives effect on mucosal microbiota. It has the chance of improving medicinal measure for mounting gut disease-based bacteria. Mushroom polysaccharides are eventually important prebiotics, which have different bioactive mechanisms of oligosaccharides. The consequences of prebiotic ingestion work in a different way. If the composition of the gastrointestinal microbiota changes, prebiotic shows its positive effect. The beneficial bacteria like *Lactobacilli* and *Bifidobacteria*'s positive effects reflect the increasing number of probiotics, and it was considered as prebiotic action in gut.

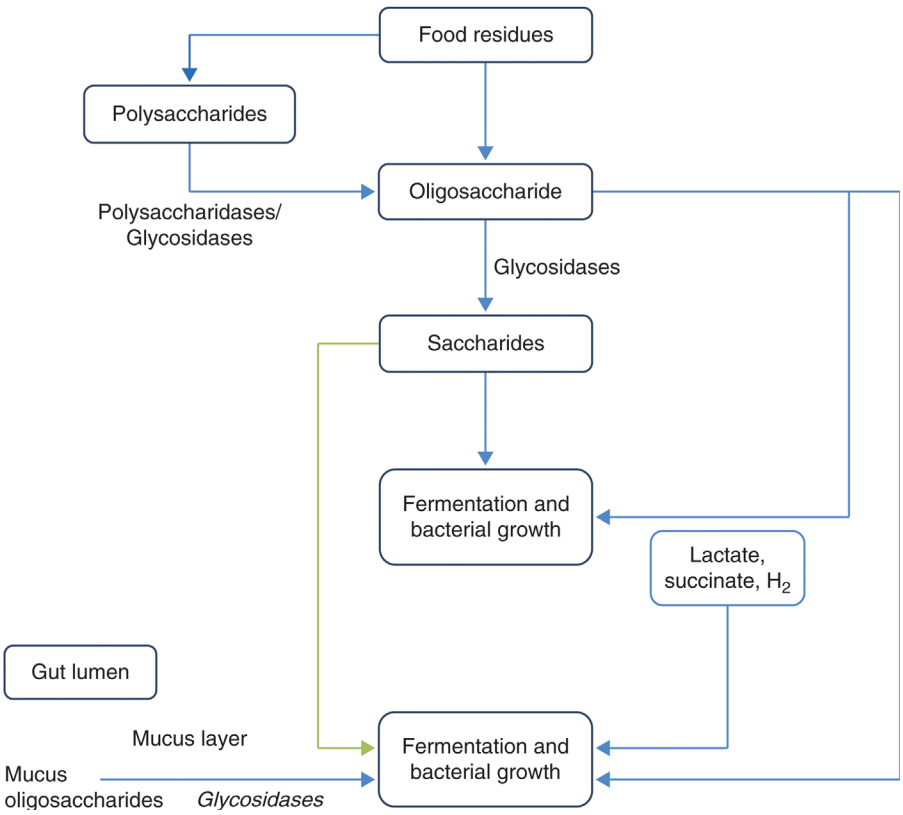


FIGURE 5.5 Mechanism of dietary food residues in the gut (Macfarlane et al., 2006).

The potential mechanism and the benefits are listed as follows:

1. In gut microbiota components, the effects of prebiotics are available through competitive substrates.
2. The regulations of carbohydrate dynamic process are utilized through certain microbiota composition.
3. Increment in epithelial cell's barrier function.
4. Vitamin production and available modulations of gut microbiota.
5. Stimulates human innate immune response.

1.13 Potential immunomodulatory mechanism of prebiotics

Polymeric macromolecule considering polysaccharides has various linkage manners that form monosaccharides. It can attach with a higher value when compared to nucleic acids and proteins. The resources of new polysaccharide and its applications of mushroom are discovered in past years. The properties and functions of mushroom polysaccharides have been investigated and estimated. Prebiotics are deliberated by the colonic integrity improvement, which increase immune function, lower LCFAs in bowels, and reduce the period of infections in intestine. The mechanism of biological application is still ill defined, and it is needed to do some work for its applications. For industrial applications, biological property and research study are needed for further analysis in future. The prebiotic mechanisms of prebiotics are listed in Table 5.3.

1.14 Gastrointestinal effects of prebiotics

Prebiotics are the foods of probiotics. The effect of prebiotics is positive and its being alters the gut microbiota composition in the way that supports the growth of probiotics such as *Bifidobacteria* and *Lactobacilli* that suppress the growth of undesired bacteria, lower the incidence of infection, improve colonic integrity, and enhance host immunity (Macfarlane

TABLE 5.3 Major prebiotics benefits and potential mechanisms of action (Vieira et al., 2013).

Prebiotic	Medical/clinical benefits	Mechanisms of action
Inulin	Crohn's disease	Promote immune response
	Colitis	Induce on innate immunity
	Obesity Colon cancer Diabetes type 2 Constipation	Alternation of microbiota and increase in *Bifidobacteria*
FOS (fructo-oligosaccharides)	Crohn's disease	Increase in *Bifidobacteria*
	Colitis	Decrease in colon pH
	Obesity	Lowering in lipid accumulation
	Constipation	Antiinflammatory substances secretion
	Travelers' diarrhea	Local induction of reactive oxygen species (ROS)
	Colon cancer	
GOS (galacto-oligosaccharides)	Crohn's disease	Improve the action of growth performance and immune responses
	Colitis	Decrease intestinal bacteria over growth
	Obesity	
Soluble fiber (Guar gum, pectin)	Crohn's disease	Growth of short-chain fatty acid production and acetate
	Celiac disease	Intestinal microbiota standardized
	Colitis	Effects on epithelial permeability
	Colon cancer	Trophic effects on enterocytes
	Metabolic syndrome	Antiinflammatory effects
	Arthritis	Increase immune response
	Cardiovascular diseases	Lower blood pressure and LDL serum concentrations

et al., 2008). Gut microbiota is not only important for gut health but is also associated with several metabolic dysregulations, which could lead to inflammatory diseases such as diabetes, obesity, and cancer (Jayachandran et al., 2017). It has been showing that gut microbiota is essential to regulate immune homeostasis in the host, which affect both the innate and adaptive immunity (Wu & Wu, 2012).

Prebiotic is a new concept for humans in their digestive function and nutrition supplement. The health and physiological claims were made already. The claims are listed in the following.

The microflora effects emphasize the colonization resistance strength for reducing the diarrheal diseases and pathogenic invasion in a large bowel. The gastrointestinal effects are more important benefits of fermentation. As demonstrated in animal models, prebiotics share lots of beneficial effects such as mineral absorption, nonstarch polysaccharides, resistant starch, and protecting tumor growth by calcium. Prebiotic research activates the immune system in two ways: (1) provoking the innate immune system directly or (2) promoting the improvement of commensal microbiota (Kühlwein et al., 2013, 2014).

1.15 Effects of prebiotic in gastrointestinal

- Large bowel fermentation (Cummings & Macfarlane, 2002)
  1. Production of SCFAs and lactate
  2. Gas, mainly CO_2 and H_2
  3. Biomass increase
  4. Improved fecal energy and nitrogen
  5. Mild laxative properties
- On the microflora
  1. Selective increases in *Bifidobacteria* and *Lactobacilli* in planktonic and biofilm communities
  2. Reduction of *Clostridia*
  3. Increase in colonization against pathogens
  4. Potential benefit in preventing pathogen invasion
- Small intestine
  1. Low-molecular-weight prebiotics
  2. Absorption of calcium, magnesium, and iron increased
  3. Interaction with mucus to change binding sites for bacteria, lectins, etc.
- Mouth
  1. Protection against caries
- Other effects
  1. Metabolism of bile acid
  2. Microbial enzymes with variable effects
  3. Apoptosis stimulation

1.16 Mushrooms as a promising prebiotic

Prebiotics are the substance that influences the microorganism's growth or action in human gut. The role of mushrooms improves the host health. Recently, new prebiotic alternatives have been gaining attention from different materials of plants such as chicory root, cereals, soya beans, potatoes, herbs, and citrus (Chou et al., 2013; Gullón et al., 2013). Apart from plant materials, mushrooms polysaccharides are considered as a potential prebiotic. The prebiotics are identified in fibers. The commonly used prebiotic raw foods are chicory root, Jerusalem artichoke, dandelion greens, garlic, and onion. Apart from these prebiotic foods, mushrooms act as a high potential emergence of prebiotics and thereby enhance the colonization integrity of the host gut and resistant to potential pathogens (Guo et al., 2003).

Furthermore, some researches have indicated that polysaccharide containing mushroom such as *L. edodes*, *Tremella fuciformis* (Guo et al., 2004), *Pleurotus* sp. (Synytsya, Jablonsky, Jana, & Kovár, 2009), and *Agaricus bisporus* (Giannenas et al., 2010) showing prebiotic activity. The active components are believed to be the long-chain beta glucans, homo-, and hetero-glucans with β (1→3), β (1→4), and β (1→6) glucosidic linkages (Manzi & Pizzoferrato, 2000).

Prebiotic's concept is carried out by some ideal criteria that include gastric acidity tolerance, intestinal microflora fermented and action of selective stimulation, absorption of mammalian enzymes and gastrointestinal, and the intestinal bacteria activity connected with health and host. The prebiotic criteria are illustrated in Fig. 5.7. Prebiotic substance indicates that the upper gut tract is repellent and is processed digestive before they reach colon. Hence, the effect is stimulated by

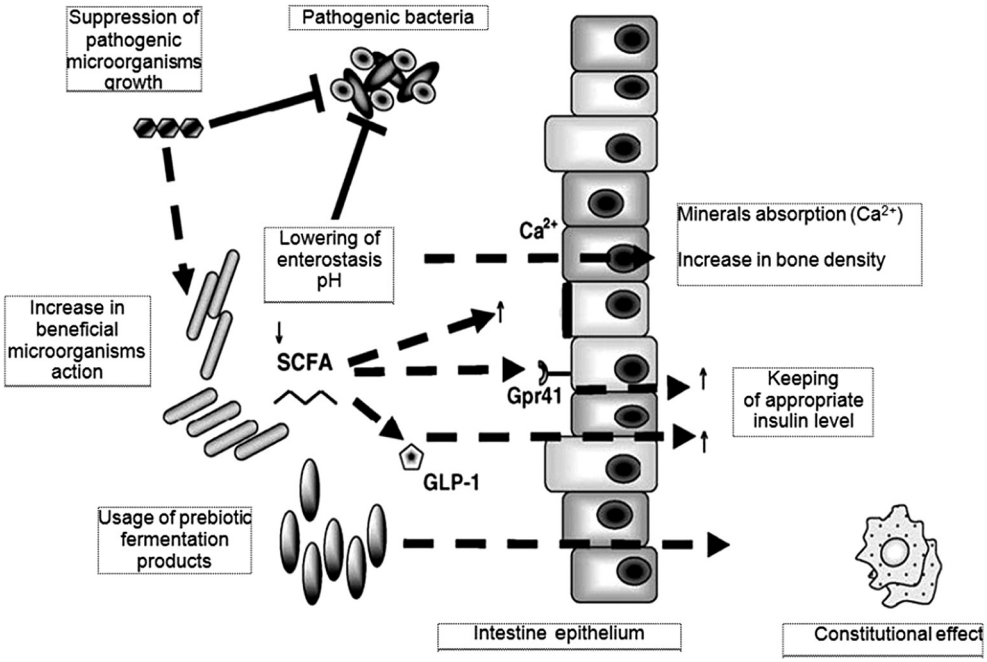


FIGURE 5.6 Mechanism action of prebiotics (Singdevsachan et al., 2016).

the beneficial bacteria like *Bifidobacteria* and *Lactobacilli*. It shows in first attributes (Wang, 2009). The fermentation of prebiotic can be potentially done with the help of beneficial bacteria in the colon. So, the prebiotic intake gives many positive benefits to health. Prebiotic food ingredient allows the criteria that comprise the potentially beneficial bacteria through selective fermentation in the colon.

The intake of prebiotic increases colonic integrity, promotes immune function, lowers the infections in intestine, reduces pH values in bowels, degrades LCFAs in bowels, and upgrades the production of SCFAs in downregulated allergic responses. Besides, it helps as boost digestion and excretion of feces. However, the fermentation effects lead to an increase in the expression or change in the composition of SCFAs (Fig. 5.6). The most important positive effect contributed by prebiotics is significant in the growth of probiotics such as *Bifidobacteria* and *Lactobacilli* (Śliżewska, KapuŚniak, Barczyńska, & Jochym, 2012).

Prebiotic ferments the colon by potentially beneficial bacteria. Fermentation plays a beneficial role to the host that leads to gain fecal weight, degrade the pH in luminal colon, increase the arrangement of SCFAs, lower nitrogenous end products and reductive enzymes, increase the expression of the binding proteins or active carriers associated with mineral absorption, and help immune system modulation (Śliżewska, KapuŚniak, Barczyńska, & Jochym, 2012). The intestinal bacteria and the activity or action of stimulation are connected with health and host. Prebiotic classification is considered as one of the typical criteria (Gibson et al., 2004). Furthermore, prebiotics supress the growth of *clostridia* and *Bacteroides* and enhance the activities of probiotics such as *Bifidobacteria* and *Lactobacilli* (Wang, 2009).

In carbohydrates, fibers and oligosaccharides are important prebiotics. It can be grouped as "established prebiotics" and "emerging prebiotics." Established prebiotics are fructooligosaccharides, galactooligosaccharides, and inulin whereas emerging prebiotics are xylooligosaccharides and isomaltooligosaccharides (Stowell, 2007). The other compounds of prebioticssuch are sorbitol, raffinose, mannitol, and maltodextrin with proven health properties (Mandal et al., 2009; Vamanu & Vamanu, 2010).

Other than fruits and vegetables, cereals, soyabeans, chiory root, oats, and herbs are major source of prebiotics. Nowadays, mushroom is acquiring much attention as an alternative root of prebiotics. Mushrooms have major constituents, which elucidate the functions of prebiotic. The polysaccharides in mushroom consist of fructose moieties that are lined by glycosidic bonds (Kelly, 2008). These polysaccharides add health benefits to the host by increasing the population of *Bifidobacteria* and *Lactobacilli* in the colon (Roberfroid, 2007; Olmstead, 2008). β-glycosidic linkage linking D-glucans is a polysaccharide, and these polysaccharides activate the population of *Bifidobacterium bifidum*, *Enterococcus*, and *Lactobacillus rhamnosus*, (Ruthes et al., 2013; Giavasis, 2014). The triple helix structures of grifloan are also polysaccharides that have beneficial effects on *Lactobacillus* and *Bifidobacterium* and adverse effects on *Salmonella*. They increase the consumption of glucose and activity of lysosomal enzyme, β-D-glucourinodase in macrophages (Thatoi et al., 2018).

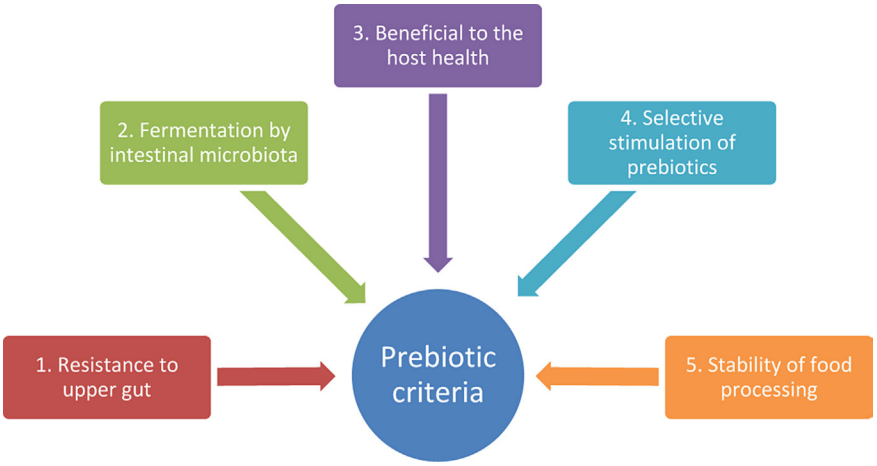


FIGURE 5.7 Criteria of prebiotics (Thatoi et al., 2018).

A potential source of mushroom polysaccharide is nondigestible substance that prevents the bacterial and viral infections in large intestine and thereby improves the growth of probiotic bacteria.

1.17 Criteria of prebiotics

Prebiotics carry out the following criteria that were proved in in vitro and in vivo tests:

1. Non-digestibleness
2. Gut microbial community of eternal fermentation
3. Enteric bacterial growth and activity

According to Wang (2009), the basic criteria of food components as a prebiotic (Fig. 5.7) include the following:

1. In the upper part of GIT, the substrate must be neither hydrolyzed nor absorbed.
2. Beneficial bacteria in the colon are potentially fermented.
3. For healthier composition, the colonic microbiota is altered.
4. Induce some effects that are useful to the host health.
5. Beneficial bacteria are selective in the colon such as bifidobacteria (Manning & Gibson, 2004).

Mushrooms such as *G. lucidum*, *Geastrum saccatum*, *Calocybe indica*, *L. edodes*, *Boletus erythropus*, *Pleurotus ostreatus*, *Hericium erinaceus*, *A. bisporus*, *F. velutipes*, and *Auricularia judae* can be used as prebiotics. All of them are effective against intestine inflammation (Zeman et al., 2001). Mushrooms containing sclerotial β-glucans utilize bacteria in human colon (Wong et al., 2005).

Crude polysaccharides are of three kinds: PSI, PSII, and PSIII. Lili and Jianchun (2008) have described the effect of these polysaccharides from *Agaricus blazei* Murill. In addition, *P. ostreatus* and *L. edodes* mushrooms influence the enteral composition of flora by elevating the partition and multiplication of useful microbes. The growth of pathogenic bacteria such as *Salmonella*, *Clostridium*, and *E. coli* are hindered (Zhou et al., 2011). Recently some edible mushrooms (*Auricularia auricula-judae*, *P. ostreatus*, *Pleurotus sajor-caju*, *Pleurotus abalonus*, and *Volvariella volvacea*) were evaluated by the prebiotic property, described by Saman et al. (2016), and the three mushrooms such as *P. ostreatus*, *P. sajor-caju*, and *Pleurotus abalones* support the growth of *Bifidobacteria* and *Lactobacilli* and could reduce the growth of harmful bacteria in human gut model.

1.18 Role of mushrooms as prebiotics

Mushrooms have a higher nutritional value with a high content of carbohydrate, proteins, vitamins, minerals, fibers, and low/no calories and cholesterol. Different kinds of mushrooms produce different types of polysaccharides that can be either water soluble or water insoluble. Prebiotic is a nondigestible food substances that influence the enhancement of microorganisms like bacteria and fungi. Based on the composition of fibers, oligosaccharides were identified as prebiotics. Generally, the prebiotic foods are present in fruits, vegetables, etc. and these foods are known as functional foods. Mushrooms

TABLE 5.4 The activity of mushrooms in the gut.

Mushrooms	Activities	References
Pleurotus eryngii	Induce the growth of *Lactobacillus* strains	Aida et al. (2009)
Pleurotus ostreatus, P. eryngii	Support the growth of colon microorganisms	Synytsya et al. (2009)
Pleurotus sajor-caju	Improve nutrient availability	Rahman et al. (2012)
Lentinus edodes, Tremella fuciformis, Agaricus bisporus	Increase intestinal microbial activities and enhance immune function	Chou et al. (2013)
Pleurotus tuber-regium, L. edodes, T. fuciformis, A. bisporus	Stimulate the growth of probiotics	Chaikliang et al. (2015)
Ganoderma lucidum	Combat obesity and obesity-related metabolic disorders	Holmes (2015)
Coriolus versicolor	Improve the growth of probiotic bacteria	Cruz et al. (2016)
P. eryngii	Modulate gut microbiota	Van Doan and Doolgindachbaporn (2016)
G. lucidum	Increase the number of *Bifidobacteria*	Van Doan et al. (2017)
Cordyceps militaris	Signify the growth and/or the activity of the bacteria	
G. lucidum, Poria cocos	Modulate gut microbiota composition	Khan et al. (2018)
G. lucidum	Raise the abundance of *Bifidobacteria*	Ma et al. (2018)
P. ostreatus, P. eryngii	Stimulate the growth rate of probiotic *Lactobacillus* bacteria	Nowak et al. (2018)
Trametes versicolor	Upgrade the growth and activity of probiotic bacteria	
Lentinus edodoes, P. eryngii	Increase inhibition and gastrointestinal tolerance	Sawangwan et al. (2018)

contain prebiotic properties with a potential source of prebiotics, which are identified as different polysaccharides, such as chitin, hemicellulose, mannans, alpha and beta glucans, galactans, proteoglycans, and xylans. It represents a critical role in antitumor and immunomodulation activities. The prebiotic mushrooms support the growth of gut microbiota in the gut, which is achieved by a host with health benefits. The prebiotics reduce the endogenic pathogens and increase the ability of immune system to refuse the exogenic pathogens (De Sousa et al., 2011). The microbiota in the gut distributes several metabolic dysregulations, leading to inflammation in the brain, liver, and intestine, and the energy metabolism is modulated. Mushroom acts as a source of potential prebiotic substrate with increased interest. The most favorable health foods of prebiotics regulate the number and structure of eternal flora (Jayachandran, Xiao & Xu, 2017). Mushrooms and their activities in the gut are mentioned in Table 5.4.

2 Conclusions

Foods were taken for dietary supplements, not only for hunger; some foods providing nutrition to health benefits are considered to be functional foods. Prebiotics are most excellent dietary substances for health-supporting, which modulates the growth and activity of specific bacterial species in the colon. The prebiotics change the microbial community composition or proportion in the lumen and at the mucosal surface. Healthier florae increase the resistance of gut infections and carry immunomodulatory properties. The health benefits of prebiotics are increased by disease resistance, which improve nutrient availability.

Prebiotics may also provide support for the survival and growth of intentionally consumed bacterial species, including some probiotic strains. We identify and characterize the bacterial genera, species, and even strains that compose the intestinal microbiota, both qualitatively and quantitatively. Food has been used as medicines in traditional medical system. Mushrooms with medicinal properties are one such kind of traditional remedy. The components present in mushrooms such as chitin, β- and α-glucans, hemicellulose, xylans, pleuran, mannans, galactans, inulin, and heteropolysaccharides enriching the prebiotic functions. Mushroom carbohydrate is a nondigestible property, which is considered as a potential source of prebiotic. The prebiotic fibers should be consistent and of adequate inclusion or dose levels to ensure desired

and continued benefits. Diet is closely related to the gut species. The intake of mushroom in a diet can potentially act as an effective prebiotic. It affects the mucosal immune system by improvement in enteral inflammatory diseases and systemic immune response directly in the gut microbiota.

Prebiotics have the potential to modify the gut microbial balance in such a way as to bring direct health benefits at low cost and safely. However, besides cereals, mushrooms are also considered as a good source of beta-glucans and have a great potential to serve as prebiotics since their world production is huge and still keeps increasing. The major concern for human health is related to dietary content and disease. Polysaccharide containing edible mushrooms indicates that the use of mushrooms as a dietary supplement is a novel approach and makes growth in nutrition, food safety, and human health.

Prebiotics have been shown to alter gastrointestinal microflora, alter the immune system, prevent colonic cancer, reduce pathogen invasion, including pathogens, and reduce cholesterol and odor compounds. Diet is only the strongest and most direct effect on gut microbial colonization because bacteria have different energy sources. In recent study, a bright new therapy for treating diseases has been multiplied. Prebiotic mushrooms in the diet increase life quality with a low risk of obesity, cancer, hypersensitivity, vascular diseases, and degenerative ailments. In future, mushrooms will be good sources for food industry for the production of a new potential prebiotic, which can be helpful for human health.

Acknowledgment

We would also like to show our gratitude to the Dr. R. Thirumurugan, Co-ordinator of National Centre for Alternatives to Animal Experiments (NCAAE) for sharing their pearls of wisdom with us during the course of this research. The financial assistance of the National Centre for Alternatives to Animal Experiments (NCAAE), under University Grand Commission Centre with Potential for Excellence in Particular Area (UGC-CPEPA) and the Department of Science and Technology, Government of India, under DST-Promotion of University Research and Scientific Excellence (PURSE) scheme-Phase II, Rashtriya Uchchatar Shiksha Abhiyan (RUSA)-2.O.

References

Aida, F. M. N. A., Shuhaimi, M., Yazid, M., & Maaruf, A. G. (2009). Mushroom as a potential source of prebiotics: A review. *Trends in Food Science & Technology*, *20*, 567–575.

Akindahunsi, A. A., & Oyetayo, F. L. (2006). Nutrient and antinutrient distribution of edible mushroom, *Pleurotus tuber-regium* (Fries) Singer. *LWT Food Science & Technology*, *39*, 548e553.

Barile, D., & Rastall, R. A. (2013). Human milk and related oligosaccharides as prebiotics. *Current Opinion in Biotechnology*, *24*, 6–11.

Bode, L. (2009). Human milk oligosaccharides: Prebiotics and beyond. *Nutrition Review*, *67*(Suppl. 2), S183–S191.

Borchers, A. T., Stern, J. S., Hackman, R. M., Keen, C. L., & Eric Gershwin, M. (1999). Mushrooms, tumors, and immunity (44412). *Proceeding of the Society for Experimental Biology and Medicine*, *221*, 281–293.

Chaikliang, C., Wichienchot, S., & Youravoug, W. (2015). Evaluation on prebiotic properties of β-glucan and oligo-β-glucan from mushrooms by human fecal microbiota in fecal batch culture. *Functional Foods in Health and Disease*, *5*(11), 395–405.

Chandrasekaran, G., Oh, D. -S., & Shin, H. (2011). Properties and potential applications of the culinary-medicinal cauliflower mushroom, *Sparassis crispa* Wulf.:Fr. (Aphyllophoromycetideae): A review. *International Journal of Medicinal Mushrooms*, *13*(2), 177–183.

Chou, W., Sheih, I., & Fang, T. J. (2013). The applications of polysaccharides from various mushroom wastes as prebiotics in different systems. *Journal of Food Science*, *78*(7), M1041–M1048.

Cruz, A., Pimentel, L., Rodríguez-alcalá, L. M., Fernandes, T., & Pintado, M. (2016). Health benefits of edible mushrooms focused on *Coriolus versicolor*: A review. *Journal of Food and Nutrition Research*, *4*(12), 773–781.

Cui, L., Yan, C., Li, H., Kim, W., Hong, L., Kang, S., et al. (2018). A new method of producing a natural antibacterial peptide by encapsulated probiotics internalized with inulin nanoparticles as prebiotics. *Journal of Microbiology & Biotechnology*, *28*, 510e9.

Cummings, J. H., & Macfarlane, G. T. (2002). Gastrointestinal effects of prebiotics. *British Journal of Nutrition*, *87*(Suppl. 2), S145–S151.

De Sousa, V. M. C., Dos Santos, E. F., & Sgarbieri, V. C. (2011). The importance of prebiotics in functional foods and clinical practice. *Food Nutrition Science*, *2*, 4.

Douglas, L. C., & Sanders, M. E. (2008). Probiotics and prebiotics in dietetics practice. *Journal of the American Dietetic Association*, *108*(3), 510–521.

Elsayed, E. A., Enshasy, H. E., Wadaan, M. A. M., & Aziz, R. (2014). Mushrooms: A potential natural source of anti-inflammatory compounds for medical applications. *Mediators of Inflammation*, *2014*, 805841.

FAO. (2020). Food & Agriculture Organization of the United Nations. Retrieved 28 June 2020, from. http://www.fao.org/faostat/en/#search/mushrooms%20and%20truffels

Ganeshpurkar, A., Kohli, S., & Rai, G. (2014). Antidiabetic potential of polysaccharides from the white oyster culinary-medicinal mushroom *Pleurotus florida* (higher Basidiomycetes). *International Journal of Medicinal Mushrooms*, *16*, 207–217.

Ganguly, S., Paul, I., & Mukhopadhayay, S. K. (2010). Application and effectiveness of immunostimulants, probiotics, and prebiotics in aquaculture: A review. *The Israeli Journal of Aquaculture—Bamidgeh*, *62*(3), 130–138.

Giannenas, I., Tontis, D., Tsalie, E., Chronis, E. F., Doukas, D., & Kyriazakis, I. (2010). Research in Veterinary Science Influence of dietary mushroom *Agaricus bisporus* on intestinal morphology and microflora composition in broiler chickens. *Research in Veterinary Science*, *89*(1), 78–84.

Giavasis, I. (2014). Bio-active fungal polysaccharides as potential functional ingredients in food and nutraceuticals. *Current Opinion Biotechnology*, *26*, 162–173.

Gibson, G. R. (2004). From probiotics to prebiotics food microbiology and safety. *Journal of Food Science*, *69*(5), M141–M143.

Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the colonic microbiota: Introducing the concept of prebiotics. *Journal of Nutrition*, *125*, 1401–1412.

Gibson, G. R., Probert, H. M., Rastall, R. A., & Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. *Nutrition Research Reviews*, *17*, 259–275.

Guggenheim, A. G., Wright, K. M., & Zwickey, H. L. (2014). Immune modulation from five major mushrooms: Application to integrative oncology. *Integrated Medicines (Encinitas)*, *13*, 32–44.

Guillamon, E., Garcia-Lafuente, A., Lozano, M., D'Arrigo, M., Rostagno, M. A., Villares, A., et al. (2010). Edible mushrooms: Role in the prevention of cardiovascular diseases. *Fitoterapia*, *81*, 715–723.

Gullón, B., Gómez, B., Martínez-Sabajanes, M., Yáñez, R., Parajó, J. C., & Alonso, J. L. (2013). Pectic oligosaccharides: manufacture and functional properties. *Trends in Food Science & Technology*, *30*(2), 153–161.

Guo, F. C., Williams, B. A., Kwakkel, R. P., & Verstegen, M. W. A. (2003). In vitro fermentation characteristics of two mushroom species, an herb, and their polysaccharide fractions, using chicken cecal contents as inoculum. *Poultry Science*, *82*, 1608–1615.

Guo, F. C., Williams, B. A., Kwakkel, R. P., Li, H. S., Li, X. P., Luo, J. Y., et al. (2004). Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. *Poultry Science*, *83*, 175–182.

Holmes, D. (2015). Medicinal mushroom reduces obesity by modulating microbiota. *Nature Publishing Group*, *11*(9), 504.

Huebbe, P., Nikolai, S., Schloesser, A., Herebian, D., Campbell, G., Glüer, C. -C., et al. (2017). An extract from the Atlantic brown algae *Saccorhiza polyschides* counteracts diet-induced obesity in mice via a gut related multi-factorial mechanism. *Oncotarget*, *8*, 73501–73515.

Jayachandran, M., Xiao, J., & Xu, B. (2017). A critical review on health promoting benefits of edible mushrooms through gut microbiota. *International Journal of Molecular Science*, *18*, 1–12.

Kelly, G. (2008). Insulin type prebiotics. A review: Part 1. *Alternative Medicine Review*, *13*, 15.

Khan, I., Huang, G., Li, X., Leong, W., Xia, W., & Hsiao, W. L. W. (2018). Mushroom polysaccharides from *Ganoderma lucidum* and *Poriacocos* reveal prebiotic functions. *Journal of Functional Foods*, *41*, 191–201.

Khangwal, I., & Shukla, P. (2019). Potential prebiotics and their transmission mechanisms: Recent approaches. *Journal of Food and Drug Analysis*, *27*, 649–656.

Kong, Q., Dong, S., Gao, J., & Jiang, C. (2016). In vitro fermentation of sulfated polysaccharides from *E. prolifera* and *L. japonica* by human fecal microbiota. *International Journal of Biological Macromolecules*, *91*, 867–871.

Kosanic, M., Rankovic, B., & Dasic, M. (2012). Mushrooms as possible antioxidant and antimicrobial agents. *Iranian Journal of Pharmaceutical Research*, *11*, 1095–1102.

Kühlwein, H., Emery, M. J., Rawling, M. D., Harper, G. M., Merrifield, D. L., & Davies, S. J. (2013). Effects of a dietary β-(1,3) (1,6)-D-glucan supplementations on intestinal microbial communities and intestinal ultrastructure of mirror carp (*Cyprinus carpio* L.). *Journal of Applied Microbiology*, *115*, 1091–1106.

Kühlwein, H., Merrifield, D. L., Rawling, M. D., Foey, A. D., & Davies, S. (2014). Effects of dietary β-(1,3)(1,6)-D-glucan supplementation on growth performance, intestinal morphology and haemato-immunological profile of mirror carp (*Cyprinus carpio* L.). *Journal of Animal Physiology & Animal Nutrition*, *98*, 279–289.

Lam, K., & Cheung, P. C. (2013). Non-digestible long chain beta-glucans as novel prebiotics. *Bioactive Carbohydrates and Dietary Fibre*, *2*(1), 1–90.

Lili, Y., & Jianchun, H. (2008). Effect of *Agaricus blazei* Murill polysaccharides on lactic acid bacteria. *China Dairy Industry*, *12*, 7–10.

Loria-Kohen, V., Lourenço-Nogueira, T., Espinosa-Salinas, I., Marín, F. R., Soler-Rivas, C., & de Molina, A. R. (2014). Nutritional and functional properties of edible mushrooms: a food with promising health claims. *Journal of Pharmacy and Nutrition Sciences*, *4*, 187–198.

Ma, G., Yang, W., Zhao, L., Pei, F., Fang, D., & Hu, Q. (2018). A critical review on the health promoting effects of mushrooms nutraceuticals. *Food Science and Human Wellness*, *7*, 125–133.

Macfarlane, G. T., Steed, H., & Macfarlane, S. (2008). Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. *Journal of Applied Microbiology*, *104*, 305–344.

Macfarlane, S., Macfarlane, G. T., & Cummings, J. H. (2006). Review article: prebiotics in the gastrointestinal tract. *Alimentary Pharmacology & Therapeutics*, *24*, 701–714.

Mallik, B. P. (2018). Evaluation of prebiotic score of edible mushroom extract. *International Journal of Engineering Research & Technology (IJERT)*, *7*(10), 122–127.

Mandal, V., Sen, S. K., & Mandal, N. C. (2009). Effect of prebiotics on bacteriocin production and cholesterol lowering activity of *Pediococcus acidilactici* LAB 5. *World Journal of Microbiology and Biotechnology*, *25*(10), 1837–1841.

Manning, T. S., & Gibson, G. R. (2004). Prebiotics. *Best Practice & Research Clinical Gastroenterology*, *18*(2), 287–298.

Manzi, P., & Pizzoferrato, L. (2000). Beta-glucans in edible mushrooms. *Food Chemistry*, *68*(3), 315–318.

Martel, J. (2017). Immunomodulatory properties of plants and mushrooms. *Trends in Pharmacological Sciences*, *38*, 967–981.

Mattila, P., Konko, K., Eurola, M., Pihlava, J. M., Astola, J., Vahteristo, L., et al. (2001). Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. *Journal of Agricultural and Food Chemistry*, *9*(5), 2343–2348.

Mattila, P., Salo-Vaananen, P., Konko, K., Aro, H., & Jalava, T. (2002). Basic composition and amino acid contents of mushrooms cultivated in Finland. *Journal of Agricultural and Food Chemistry*, *50*(22), 6419–6422.

Monali, B., & Kumar, P. (2018). Mushroom polysaccharides as a potential prebiotic. *International Journal of Health Science Research*, *3*(8), 77–84.

Musatto, S. T., & Mancilha, I. M. (2007). Non-digestible oligosaccharides: A review. *Carbohydrate Polymers*, *68*, 587–597.

Nowak, R., Nowacka, N., Marek, J., & Anna, J. (2018). The preliminary study of prebiotic potential of polish wild mushroom polysaccharides: The stimulation effect on *Lactobacillus* strains growth. *European Journal of Nutrition*, *57*, 1511–1521.

Olmstead, S. (2008). Understanding prebiotics. The current trends and future perspectives of prebiotic research: A review. *Biotechnology*, *2*, 115–125.

Pandey, K. R., Naik, S. R., & Vakil, B. V. (2015). Probiotics, prebiotics and synbiotics—A review. *Journal of Food Science & Technology*, *52*(12), 7577–7587.

Rahman, A. B. D. J. M. D., Abdul Razak, S., & Sabaratnam, V. (2012). Effect of mushroom supplementation as a prebiotic compound in super worm based diet on growth performance of red tilapia fingerlings. *Sains Malaysiana*, *41*(10), 1197–1203.

Rao, J. R., Millar, B. C., & Moore, J. E. (2009). Antimicrobial properties of shiitake mushrooms (*Lentinula edodes*). *International Journal of Antimicrobial Agents*, *33*, 591–592.

Rathore, H., Prasad, S., & Sharma, S. (2017). Mushroom nutraceuticals for improved nutrition and better human health: A review. *Pharma Nutrition*, *5*(2), 35–46.

Ringo, E., & Olsen, R. E. (1999). The effect of diet on aerobic bacterial flora associated with intestine of Arctic charr, *Salvelinus alpinus* (L.). *Journal of Applied Microbiology*, *88*, 22–28.

Ringo, E., Olsen, R. E., Gifstad, T. O., Dalmo, R. A., Amlund, H., Hemre, G. -I., et al. (2010). Pebiotics in aquaculture: A review. *Aquaculture Nutrition*, *16*, 117–136.

Roberfroid, M. (2007). Prebiotics: The concept revisited. *The Journal of Nutrition*, *137*(3), 830S–837S.

Ruthes, A. C., Rattmann, Y. D., Malquevicz, P. S. M., Carbonero, E. R., Cordov, M. M., & Baggio, C. (2013). *Agaricus bisporus* fucogalactan: Structural characterization and pharmacological approaches. *Carbohydrate Polymers*, *92*, 184–191.

Samal, L., & Behura, N. C. (2018). Prebiotics: An emerging nutritional approach for improving gut health of livestock and poultry. *Asian Journal of Animal and Veterinary Advances*, *10*(11), 724–739.

Saman, P., Chaiongkarn, A., Moonmangmee, S., Sukcharoen, J., & Kuancha, C. (2016). Evaluation of prebiotic property in edible mushrooms. *Biological and Chemical Research*, *3*, 75–85.

Sawangwan, T., Wansanit, W., & Pattani, L. (2018). Study of prebiotic properties from edible mushroom extraction. *Agriculture and Natural Resources*, *52*(6), 519–524.

Schrezenmeir, J., & Michael, V. (2001). Probiotics, prebiotics and synbiotics – Approaching a definition. *American Journal of Clinical Nutrition*, *73*(Suppl.), 361–364.

Singdevsachan, S. K., Auroshree, P., Mishra, J., Baliyarsingh, B., Tayung, K., & Thatoi, H. (2016). Mushroom polysaccharides as potential prebiotics with their antitumor and immunomodulating properties: A review. *Bioactive Carbohydrates and Dietary Fibre*, *6198*(15), 1–14.

Singla, V., & Chakkaravarthi, S. (2017). Applications of prebiotics in food industry: A review. *Food Science and Technology International*, *23*(8), 649–667.

Siqian, L., & Shah, N. P. (2015). Effects of *Pleurotus eryngii* polysaccharides on bacterial growth, texture properties, proteolytic capacity, and angiotensin I converting enzyme inhibitory activities of fermented milk. *Journal of Dairy Sciences*, *98*, 2949–2961.

Śliżewska, K., KapuŚniak, J., Barczyńska, R., & Jochym, K. (2012). In C. -F. Chang (Ed.), *Comprehensive studies on glycobiology and glycotechnology* (pp. 261–288). Rijeka, Croatia, Europe: InTech.

Solano-Aguilar, G. I., Jang, S., Lakshman, S., Gupta, R., Sikaroodi, M., Vinyard, B., et al. (2018). The Effect of dietary mushroom *Agaricus bisporus* on intestinal microbiota composition and host immunological function. *Nutrients*, *10*, 1–16.

Stowell, J. (2007). In H. Mitchell (Ed.), *Calorie control and weight management* (pp. 54–64). Oxford, United Kingdom: Blackwell Publishing Ltd.

Synytsya, A., Jablonsky, I., Jana, C., & Kovár, E. (2009). Glucans from fruit bodies of cultivated mushrooms *Pleurotus ostreatus* and *Pleurotus eryngii*: Structure and potential prebiotic activity. *Carbohydrate Polymers*, *76*, 548–556.

Taofiq, O., González, P. A. M., Martins, A., Barreiro, M. F., & Ferreira, I. C. F. R. (2016). Mushrooms extracts and compounds in cosmetics, cosmeceuticals and nutricosmetics: A review. *Industrial Crops and Products*, *90*, 38–48.

Thatoi, H., Singdevsachan, S. K., & Patra, J. K. (2018). Prebiotics and their production from unconventional raw materials (Mushrooms). *Therapeutic, Probiotic, and Unconventional Foods*, 79–99.

Toghyani, M., Tohidi, M., Gheisari, A., Tabeidian, A., & Toghyani, M. (2012). Evaluation of Oyster mushroom (*Pleurotus ostreatus*) as a biological growth promoter on performance, humoral immunity, and blood characteristics of broiler chicks. *Journal of Poultry Science*, *49*, 183–190.

Valverde, M. E., Hernández-pérez, T., & Paredes-lópez, O. (2015). Edible mushrooms: Improving human health and promoting quality life. *International Journal of Microbiology*, *2015*, 1–14.

Vamanu, E., & Vamanu, A. (2010). The influence of prebiotics on bacteriocin synthesis using the strain *Lactobacillus paracasei* CMGB16. *African Journal of Microbiology Research*, *4*(7), 534–537.

Van Doan, H., & Doolgindachbaporn, S. (2016). Effects of Eryngii mushroom (*Pleurotus eryngii*) and *Lactobacillus plantarum* on growth performance, immunity and disease resistance of Pangasius catfish (*Pangasius bocourti*, Sauvage 1880). *Fish Physiology and Biochemistry*, *42*(5), 1427–1440.

Van Doan, H., Hossein, S., Dawood, M. A. O., Chitmanat, C., & Tayyamath, K. (2017). Effects of *Cordyceps militaris* spent mushroom substrate and *Lactobacillus plantarum* on mucosal, serum immunology and growth performance of Nile tilapia (*Oreochromis niloticus*). *Fish and Shellfish Immunology*, *70*, 87–94.

Vieira, A. T., Teixeira, M. M., & Martins, F. S. (2013). The role of probiotics and prebiotics in inducing gut immunity. *Frontiers in Immunology*, *4*(445), 1–12.

Wang, Y. (2009). Prebiotics: Present and future in food science and technology. *Food Research International*, *42*(1), 8–12.

Wasser, S. P. (2002). Medicinal mushrooms as a source of antitumor and immunomodulatory polysaccharides. *Application of Microbiological Biotechnology*, *60*, 258–274.

Wong, K. H., Wong, K. Y., Kwan, H. S., & Cheung, P. C. (2005). Dietary fibres from mushroom sclerotia: 3. *In vitro* fermentability using human fecal microflora. *Journal of Agricultural and Food Chemistry*, *53*(24), 9407–9412.

Wu, H. -J., & Wu, E. (2012). The role of gut microbiota in immune homeostasis and autoimmunity. *Gut Microbes*, *3*(1), 4–14.

Yamin, S., Shuhaimi, M., Arbakariya, A., Fatimah, A. B., Khalilah, A. K., Anas, O., et al. (2012). Effect of *Ganoderma lucidum* polysaccharides on the growth of *Bifidobacterium* spp. as assessed using real-time PCR. *International Food Research Journal*, *19*, 1199–1205.

Zaidman, B., Yassin, M., Mahajana, J., & Wasser, S. P. (2005). Medicinal mushroom modulators of molecular targets as cancer therapeutics. *Applied Microbiology Biotechnology*, *67*, 453–468.

Zeman, M., Nosalova, V., Bobek, P., Zakálová, M., & Černá, S. (2001). Changes of endogenous melatonin and protective effect of diet containing pleuran and extract of black elder in colonic inflammation in rats. *Biologia*, *56*, 659–701.

Zhang, M., Cui, S. W., Cheung, P. C. K., & Wang, Q. (2007). Antitumor polysaccharides from mushrooms: A review on their isolation process, structural characteristics and antitumor activity. *Trends in Food Science & Technology*, *18*, 4–19.

Zhou, B. -L., Liang, Q., Zou, Y., Ming, J., & Zhao, G. -H. (2011). Research progress in prebiotic properties of edible mushroom. *Food Sciences*, *32*, 303–307.

Ziemer, C. J., & Gibson, G. R. (1998). An overview of probiotics, prebiotics and synbiotics in the functional food concept: Perspectives and future strategies. *International Dairy Journal*, *8*, 473–479.

Part II

Omics approaches in Probiotics

6. Genetic Modification and Sequence Analysis of Probiotic Microorganisms 101
7. Biosynthetic Gene Cluster Analysis in *Lactobacillus* Species Using antiSMASH 113
8. Probiotic Polysaccharides as Toll-Like Receptor 4 Modulators—An In Silico Strategy 121

Chapter 6

Genetic Modification and Sequence Analysis of Probiotic Microorganisms

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1 Introduction

The microbiome is a term that defines the genomes of all symbiotic, commensal, or pathogenic microorganisms living in a particular environment (Lederberg & McCray, 2001). Microbiota is defined as all microorganisms that live together in a particular environment. These two terms are generally used as synonyms, but as they can be understood from their definition, they mean different things. In summary, the term microbiome refers to the microorganisms and their genes, while the term microbiota refers to the taxonomy of microorganisms (Hemarajata & Versalovic, 2013). Microbiome analysis, which covers a broader framework than microbiota, has a high potential to identify interactions between microorganisms and their host and its genetic and physiological results. Accordingly, in order to understand the genetic and physiological diversity in humans, the necessity of determining the factors affecting the evolution with the distribution of the microbiome and the microorganisms forming the microbiome formed the main idea of the human microbiome project (HMP). The HMP is one of the largest biological projects ever conducted. Launched in 2008 by the National Institute of Health, this project has allocated a budget of $150 million for 5 years. In this project, analyses were carried out in five different body regions, namely, skin, nose, mouth, gut, and vagina of healthy individuals. When the project was completed in 2013, 14 TB of data were produced as a result of the analyses performed in 300 individuals (Radilla-Vázquez, Parra-Rojas, & Martínez-Hernández, 2016; Gomes, Costa, & Alfenas, 2017). Although with the integrative HMP (iHMP) project studies to catalog the genes in the large intestine produce 42 TB of data, it is still far from identifying the core microbiome (Cani, 2018). To date, microbial gene content has been sampled in approximately 800 healthy people. These studies are still going on in hundreds of people. In other words, the depth of microbiota measurements obtained by the HMP by 2020 is yet to reveal what constitutes a healthy microbiome. The results demonstrate that the "healthy microbiota" approach has its limitations. Since mass cataloging of microbial species and genes still cannot reveal what represents a balanced microbial community, some scientists suggest that the concept of equilibrium is immeasurable and, therefore, not useful for microbiome research (Gomes et al., 2017). Despite all this fact, microbiome analyses are still the most common way to define host-microorganism relationships.

Unlike the microbial composition, the metagenomic genome data show a relatively uniform distribution and prevalence of metabolic pathways across body sites and individuals. Dominant metabolic modules such as central carbohydrate metabolism pathways have been identified to represent relevant functions of the distal small intestine and other body regions. However, 86% of gene families identified from intestinal metagenomes have not yet been functionally characterized or perfectly matched to metabolic pathways (Huttenhower et al., 2012; Methe et al., 2012). In summary, there is still a long way to go in determining both microbiota components and their functions within the host system.

Prokaryotic and eukaryotic microorganisms that make up the microbiota are bacteria, viruses, protozoa, yeast, and molds. The ground-breaking developments in metagenomics over the past 20 years have offered insights into microbial diversity in the human gut system. On the one hand, studies on the microbiome have allowed individual microbiota to be

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00006-X
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defined and classified in detail. They provide us with invaluable information about the evolutionary origin of host/microorganisms relationships. The gastrointestinal system is the most extensive organ system of host/microorganisms relationship and ecological relationships between microorganisms. Through ecological studies, it is possible to define all types of relationships between biotic and abiotic things and their environment (Mu, Yang, & Zhu, 2016; Jie et al., 2017; Cani, 2018; Nishino et al., 2018).

On the other hand, whole-genome sequencing (WGS) of probiotic microorganisms, in addition, to determine the genetic composition of the organisms in question, including single nucleotide polymorphism variants, insertion and deletions, copy number variations, and large structural variants (SV), is also possible to define (Mu, Yang, & Zhu, 2016; Cani, 2018; Nishino et al., 2018). Today, it has been possible to provide detailed data regarding the genetic, physiological, and evolutionary origin of probiotics by using whole-genome sequence analyses, functional genomic analysis, and other omic techniques. Integrated genomic, transcriptomic, proteomic, and secretomic studies carried out in this direction have led to a serious way of defining the interactions between probiotics and the human gastrointestinal system. In addition to the genomic, transcriptomic, metabolomic, and secretomic data obtained within the framework of the studies described in detail earlier, the developments in gene cloning and genome editing made the construction of genetically modified probiotic strains are possible.

2 Sequence analyses

Rather than core microbial communities, disruptions in core microbial functions are more associated with changes in physiological or disease states. Unfortunately, we are still not capable of cultivating approximately 95% of microbial species. Therefore, instead of individual DNA sequencing analysis of microbial genomes, metagenomic approaches based on the analysis of the genes of microbial communities have been developed. The first metagenomic studies on human microbial communities were conducted using the traditional Sanger platform. Especially after the discovery of the pyrosequencing method, the use of 16S-based data sets has increased very rapidly. The use of 16S rRNA targeted amplicon sequencing contained some limits in the microbial classification at the genus or operational taxonomic units level, which led to the requirement to develop more advanced descriptive methods, especially at the species level. One of these most preferred methods is the ultra-deep metatranscriptomic shotgun sequencing method.

Comparative metagenomics plays an essential role in explaining these relationships by using databases such as the Kyoto Gene and Genome Encyclopedia (KEGG), and Ortholog Group Clusters (COG). KEGG is a database source containing genomic, chemical, and systemic functional information necessary to understand the biological functions of genes involved in cellular networks. COG, on the other hand, is a database based on phylogenetic analysis of proteins encoded by complete genomes in bacteria, archaea, and eukaryotes. Findings from these integrative studies have shown that some genetic components of microorganisms in the gut microbiota can perform some functions that the human gut needs, but they are not encoded in the human genome. Genes encoding functions that play a role in polysaccharide metabolism (Pflughoeft & Versalovic, 2012), metanogenic pathways for hydrogen gas removal, and enzymes necessary for the detoxification of xenobiotics (Fukoi, 2019) are some of the functional genetic categories defined in intestinal microbial communities. As a result of analyses carried out in the human intestine, up to 3 Gb microbial sequence was produced from fecal samples of 33 people from the United States and Japan. In another study, 576.7 sequences were generated as a result of deep DNA sequencing of DNA samples isolated from the feces of 124 European individuals. The latest data from the HMP show that this number is more than 5.2 million (Huttenhower et al., 2012; Methe et al., 2012). The International Human Microbiome Consortium predicts that the total genes will be more than 8 million. This gene set is 300 times larger than all the genes in the human genome. The gene set contains a core set consisting of different enzyme families, most of which are essential for microbial life. It may be limited to 24 of the metabolic and functional modules expressed in all samples (Abubucker et al., 2012). Scientists are currently researching gene expression patterns in the human microbiome to understand and interpret these metagenomic data. A meta-transcriptomic analysis performed using cDNA (complementary DNA) libraries prepared from fecal samples of healthy volunteers has confirmed the presence of common microbiota genes that play a role in carbohydrate metabolism, energy production, and synthesis of cellular components (Gosalbes et al., 2011).

Intestinal microorganisms can alter gene expression in the mammalian intestinal mucosa and ultimately affect the function of the gastrointestinal tract. In a study conducted using sterile mouse models, intestinal microbiota was shown to modulate the expression of many genes in the human or mouse gut system, including genes involved in the immune system, nutrient absorption, energy metabolism, and gut barrier functions (Larsson et al., 2012). Another exciting finding identified in this study was that most of these gene expression changes occur in the mucous membrane of the small intestine. The presence of probiotics in the gastrointestinal tract can also affect patterns of gene expression, as shown by another study. In one study, conducted with healthy volunteers, subjects were treated with probiotic bacteria (*Lactobacillus acidophilus* Lafti

L10, *Lb. casei* CRL-431, and *Lb. rhamnosus* GG) and esophagogastroduodenoscopy before and after a 6-week intervention period. Analysis of human gene transcription profiles in samples from subjects treated with probiotics revealed that changes occurred in transcriptional networks in the mucosal immune system. These findings are the most accurate evidence that the microbiota modulates host gene expression (Van Baarlen et al., 2010).

The intestinal microbiota is a heterogeneous community of microorganisms, and there are rare species in addition to the abundant microorganisms. Therefore, defining the core microbiome in this environment is of great importance in terms of determining the interactions between the host/microbiota. For this purpose, the diversity of the gut microbiota has been previously investigated using highly efficient rDNA sequencing (Huttenhower et al., 2012). Metagenomic analyses have shown that although more than 50 bacterial phyla have been described, in the case of intestinal microbiota, it has been observed that it is possible to limit this number to members of *Proteobacteria* and *Actinobacteria*, especially *Bacteroidetes* and *Firmicutes* (Huttenhower et al., 2012; Methe et al., 2012). It has been determined that there are approximately 57 species in the human cohort, with a rate of 90%. This number is likely to increase with further sampling. Indeed, a two- to threefold increase in sequencing depth increases the number of species that we can identify as shared between two individuals by 25%. Many of these bacteria are found in the distal part of the digestive system, especially in the large intestine, because in the proximal part, the gastric juice, bile, and pancreatic secretions are toxic or unsuitable for the growth of most microorganisms (Sommer, Anderson, Bharti, Raes, & Rosenstiel, 2017; Byndloss, Pernitzsch, & Bäumler, 2018).

With the help of metagenomic analyses, the probiotic bacterial gene set was found to be 150 times larger than the human host. In particular, priority was given to the identification of vital functions of the gut microbiota (minimal gut genome). Two main types of genes have been identified within the minimal intestinal genome. These are housekeeping genes that govern degradation of complex polysaccharides, synthesis of indispensable amino acids, vitamins, proteins, and short-chain fatty acids (Nobaek, Johansson, Molin, Ahrne, & Jeppsson, 2000). The most commonly shared gut-specific functions of members of the microbiome community were those that support interaction with the host epithelium, such as collagen, fibrinogen, and adherence to fibronectin and sugar metabolism (Pflughoeft & Versalovic, 2012). To date, a large number of bacterial genome sequencing of important probiotic strains, especially *Bifidobacterium* and *Lactobacillus*, have been completed. Genomic data for these bacteria are available in the NCBI database. The data obtained have brought new horizons and accelerated transcriptomic, proteomic, and metabolomic approaches based on probiotic genomics. Genomic analysis of probiotic bacteria (probiogenomics) plays a key role by defining the evolutionary relationships of probiotic organisms as well as determining their health-promoting activities. Based on the relationship between possible genes and probiotic functions, intensive cataloging studies are carried out. However, it is not possible to perform a functional analysis of huge data obtained from DNA sequence analyses at the same speed. These studies show much slower development than DNA sequence analysis. The vast majority of genes with functional identification as a result of probiotic genome analysis are housekeeping genes. Cataloging and functional analysis of common ancestral genes and genes acquired by horizontal gene transfer will have a serious role in both the evolution of probiotic organisms and genetic manipulation. Briefly, beyond providing the global appearance of the human microbiome, the large gene catalogs are created to guide the identification of the relationships between microbial genes and human phenotypes. These studies will lead to a complete understanding of human biology than we currently have.

3 Genetic engineering applications on probiotic strains

The application of genetically engineered microorganisms for human use is under strict regulatory control around the world. Moreover, in some countries, biosafety laws established in-line with scientific, technological, social, and ethical concerns do not allow the production of goods and services with genetic engineering techniques. Despite this, genetic engineering can be used to tackle the problem of antibiotic resistance in humans. Genetic engineering applications in probiotic bacteria are performed using its DNA or using DNA of GRAS (generally regarded as safe for human and animal consumption) microorganisms. The process can be achieved by (1) the integration of target genes into the probiotic strain or deletion of some genes by site-specific recombination using the attP/integrase system or by homologous recombination using suicide vectors; (2) the use of food-grade vectors derived from cryptic plasmids of lactic acid bacteria (LAB) and/or bifidobacteria; and (3) using the clustered regularly interspaced short palindromic repeats (CRISPR) and the CRISPR-associated (Cas) release.

3.1 Food-grade vectors

3.1.1 Cryptic plasmids

LAB has been used in food fermentations since almost when humans were organized into agricultural communities. Besides, genetic elasticities are preferred for genetic engineering applications of these bacteria. Thus, food-grade vectors are

generally derived from cryptic plasmids LAB. The presence of a replication origin in these vectors guarantees a stable replication of the plasmid. In some cases, dual replication origins are also used in these plasmids. However, in these conditions, the problem of genetic instability may arise. Another important consideration for a shuttle vector is that they contain a selective marker. The use of dominant markers and complementation markers instead of antibiotic resistance markers are basic approaches in the design of food-grade vectors. The primary dominant markers are bacteriocin resistance or immunity genes, and sugar utilization abilities, and heat-shock resistance. Complement markers are based on the creation of an auxotrophic strain in terms of essential metabolites such as amino acids, sugars, or nucleic acid precursors. In-line with the strategies described earlier, there are many cryptic plasmid vectors developed in LAB. In this way, it is aimed to prevent adverse effects caused by genetic engineering applications such as the selection of antibiotic-resistant strains and the spread of antibiotic resistance via horizontal gene transfer. The fact that antibiotic resistance markers are not legally permitted in genetically modified organisms (GMOs) has led to the design of reliable and environment-friendly food-grade vectors for human and animal consumption as well as commercial applications (Song et al., 2014; Landete, 2016, 2017).

Most of the LAB plasmids are "cryptic" plasmids, which contain the genes necessary for the plasmid replication and mobilization that have no apparent effect on the phenotype of the host. These plasmids are usually small enough to show high resistance to in vitro manipulations. The fact that they form a plasmid class commonly described in LAB is another preferred point in their use as a vector. Cryptic plasmids, usually no larger than 3 kb, contain a replication origin (*ori*), rep genes that encode the proteins required for replication, and *cop/inc* genes that participate in the control of the initiation stage of replication. Thus, the plasmid vector can perform its replication independently of the host cell, usually at a high copy number. Especially a high copy number vector is of great importance due to its segregation stability and is an indispensable element as cloning vectors. Plasmids with independent replicon mechanisms can control persistence in host cells and even horizontal gene transfer when they have a *tra* gene region (Espinosa, Cohen, & Couturier, 2000). On the other hand, the replication mode of plasmids is an important criterion that affects the stability of the vectors in question, the number of copies, and the host sequence. There are two replication models on plasmids containing LAB: sigma (rolling circle replication, RCR) and theta. RCR replicons generally show low segregation stability. The main reason for this is the accumulation of single-chain DNA intermediates in the environment. These replicons are divided into five main groups in all Gram-positive bacteria based on Rep proteins and double-chain replication origins (dso). These groups are pMV18/pE194, pC194, pT181, pSN2, and pTX14-3 (Shareck, Choi, Lee, & Miguez, 2004; Takala, Saris, & Tynkkynen, 2005). Although theta-type plasmid replicons are generally of large molecular weight and encoding industrially important properties in LAB such as lactose fermentation, protease activity, citrate fermentation, bacteriophage resistance (pSK11L, pNZ4000, pCI528, pUCL22, pSL2, etc.), these also include cryptic plasmids such as pCI305 and pWV02. Plasmids with theta-type replication mechanism are capable of carrying more extended foreign DNA inserts. Plasmid incompatibility, for mandatory protein factors in DNA replications, may occur due to competition between different plasmids with a similar replication mechanism. As a result, one of the plasmids that show incompatibility to the host cell is eliminated (because similar replication origins cannot be matched simultaneously). This elimination of selective pressure happens faster. In plasmids with rotating chain and theta-type replication mechanisms, incompatibility groups do not occur due to differences in replication mechanisms, and they can retain replication stability in the same hosts (El Demerdash, Heller, & Geis, 2003; Lahteinen et al., 2010).

Until today, many cryptic plasmids have been isolated and developed as food-grade vectors from LAB members, such as *Lactococcus lactis* (pWV01, pSH71, pD125, pLC2.1), *Lactobacillus* (pC1305, pSL2, pVS40, pWVo4, pLJ1, pCK5B, pSAK1), *Streptococcus thermophilus* (pA2, pA33, pSt04, pJ34, LeJ2, p4028, pRS1), *Pediococcus* (pRSQ1, pRSQ9, pRSQ10, pRS11, pSMB54), and *Bifidobacterium* (pAMB1, pTB6, pKJ50, pAMP1, pB44), have been developed as multiple cryptic plasmid food-grade vectors. The *nsr* gene encoding a hydrophobic protein that provides resistance to Nisin is one of the first genes used for alternative and dominant selection by the classical genetic method. For this purpose, the *nsr* (nisin resistance gene) gene was added to a cryptic pNP40 plasmid (60 kb) identified in the *L. lactis* biovariety *diacetylactis* DRC3 strain for a food-grade selection. This gene was then used as a selective marker in another lactococcal food-grade plasmid, pLEB590 vector. Another food-grade vector, pSH1, was designed as a cloning tool that contains the *nisI* (nisin immunity gene) gene and the *repA* (replication origin) gene with complete lactococcal DNA (Posno, Leer, & van Luijk, 1991; Yadav, Kumar, Baveja, & Shukla, 2018).

3.1.2 Chromosomal integration vectors

Although high success has been achieved in laboratory conditions with plasmid vector systems, most of these vectors have not been found suitable for industrial purposes. The main problem identified for these natural or recombinant plasmid vectors is bacterial mitotic disintegration stability (Posno et al., 1991). Therefore, it is often not possible to overcome segregation instability in an industrial environment or continuous fermentations. The use of criteria that create selective pressure

specific to plasmids is also an inadequate solution to this problem. Moreover, selective pressure criteria, such as antibiotics, are unlikely to be used in food applications. These problems are tried to be solved by using food-grade integrative suicide vectors and expression vectors containing regulator signals. Integrative gene cloning results in the specific insertion of a gene sequence into the chromosome of bacterial hosts without the need for antibiotics or resistant markers. The use of the intervening series (IS) is a common strategy for this purpose. Suicide vectors or recombinant vectors with temperature-sensitive genes can be developed by using the attP/integrase system placed between the IS. In the integration of vector systems into the host chromosome, recombination, both homologous and site-specific, is used. The general strategy for homologous recombination is to establish homology regions between the passenger DNA and the region intended to enter it on the chromosome, using a second DNA fragment, called guide DNA. On the other hand, the most typical example of region-specific recombination is the integration of bacteriophages into the host chromosome. This event takes place between the attachment site (*attB*) in the bacterial chromosome and the bacteriophage attachment site *(attP*) in the bacteriophage genome. Using this mechanism, food-level vectors containing *attP* regions were developed. First LAB chromosomal integration systems were created by a recombination of *int* genes and *attP* regions from *Lb. gasseri* bacteriophage Øadh, *Lb. delbrueckii* subsp. *bulgaricus* bacteriophage mV4 and *Lc. lactis subsp. lactis* subsp. *cremoris* bacteriophage Tuc2009, r1t, and ØLC3 to non-replicative plasmids. Many of the chromosomal integration vectors are suicide vectors containing heat-sensitive systems such as pTRK327, pORI, pRV300, and pJDC9 (Gosalbes, Esteban, Galan, & Perez-Martinez, 2000; Heap, Ehsaan, & Cooksley, 2012). If these vectors do not integrate into the chromosome, they do not contain independent replication ability, so they are eliminated by sequential cleavages from the cytoplasm, that is, they cannot show stability (Posno et al., 1991; Gosalbes et al., 2000; Heap et al., 2012; Song et al., 2014).

The most critical points in the integration of plasmid vectors into the host chromosome can be summarized under the following titles: (1) specificity of the series that will be integrated into the chromosome, containing homology with the integration regions; (2) the insert carrying capacity of the receiving chromosome; (3) the specificity of the recombinase enzyme systems to perform the recombination; and (4) the selected integration region suitability for chromosomal rearrangements. Most food-grade integration vectors are designed to perform "clean" recombination, that is, the gene of interest is integrated into the chromosome without affecting the genetic environment. Recombination typically takes place in noncoding regions of the chromosome. No DNA other than the target gene is added, so this approach does not interfere with the expression level of neighboring genes (clean recombination). It is generally accepted that chromosomal integration of target genes by self-cloning is safe, and these conditions provide the highest level of additional stability. Food-grade integration plasmids can be divided into two groups. These are Campbell-like integration and the two-stage homologous recombination technique. The simplest form of homologous recombination is the integration of the non-replicating vector into the host chromosome. This integration is called single crossing-over or Campbell-like crossing-over. At the end of this event, the integration of the whole vector from the locus defined in the chromosome is performed. In this way, the gene or genes in the integration region are inactivated. Usually, this is a reversible process, and therefore using Campbell-like crossing over creates stability problems. However, if there is a homology between the two regions to be recombined, double crossing-over occurs. This event also results in the chromosomal deletion and gene insertion (Peterbauer & Haltrich, 2011; Song et al., 2014). In addition to the chromosomal integration strategies described earlier, the integration of the target gene or genes into the host chromosome in multiple copies is still little studied. With the use of CIChE system, chromosomal integration of *recA*-dependent homologous recombination mediated multiple target gene copies can be achieved. In addition, flippase (FLP)-dependent recombination is another possible method for this purpose.

Providing stability of vector systems and the genes, they carry as part of the host chromosome and minimizing their interaction with other chromosomal regions is very important in terms of minimizing the unexpected effects that may arise from these applications in genetically modified probiotics. In this sense, the development of very advanced vector systems is only possible with a more detailed diagnosis of the molecular biology of plasmids, bacteriophages, and bacteria.

3.2 DNA transfer

In genetic engineering, the transfer of vectors arranged for a specific purpose to target host cells is one of the critical stages. This transfer is done through a process called transformation. Transformation can be defined as the transfer of extracellular DNA molecules into the host cell. Depending on the cell and vector type, particle bombardment, electroporation, or chemical transformation are the most commonly used laboratory-based methods in transformation. Plasmid vectors usually contain a replication origin, a selective marker, and a multiple cloning region. Food-grade plasmid replicons are usually RCR or theta type. Although RCR replicons have a wider host range, they have a lower level of stability than theta-type replicons. Although they have a narrower host range, they are more commonly used in the design of food-grade vectors due to their high stability. Plasmid DNA is inserted into the cell either outside the chromosome or integrated into the

chromosome, depending on whether the plasmid is replicative or non-replicative. To obtain transformants integrated into the chromosome, high-frequency transformation of non-replicable vectors into the selected intermediate host or direct target host strain is imperative. Because only under these conditions can chromosomal integration be achieved (Biswas, Gruss, & Ehrlich, 1993; Peterbauer & Haltrich, 2011).

In the past years, *L. lactis* has become a model organism in food-level studies due to its genetic elasticities and completion of DNA sequence analysis and its suitability for biotechnological applications. In particular, pNZ series vectors have become prominent due to their high transformation efficiency and their protein expression suitability in heterologous hosts. On the other hand, food-grade vectors of *L. lactis* origin such as pSV40, pM01, pFK012, which containing Nisin resistance, are highly useful vectors in addition to their high transfer efficiency, as well as the direct selection of transformants. Moreover, pLEB590 with a *nisI* selective marker and pLEB-derived plasmids containing the Lacticin F immunity marker were developed and presented for this purpose. Many plasmids such as pWV01, pT1NX, pNZ8048, and pSH71, isolated from *L. lactis* MG1363 or NZ9000, were found to be efficient for transformation to other homologous and heterologous host strains (Ho & Mittal, 1996; Aune & Aachmann, 2010).

When using replicative plasmids, low transformation efficiency is sufficient to transform bacteria. However, if successful results are desired for non-replicative plasmids, a thermosensitive replicon should be used in these vectors to increase transformation efficiency. This method is based on a conditional replication of vector pORI19 containing ori + repA regions derived from lactococcal plasmid pWV01. The transformation of *L. lactis* MG1363 strain containing pA6007 to express the RepA protein on the pORI19 plasmid at the permissible temperature allows the replication of several copies of the recombinant plasmid in the host cell to which it was transferred. However, with an increase in reproductive temperature (37°C), RepA is inactivated, which leads to the elimination of pOR600 in the cytoplasm (Landete, 2017).

3.3 Genetic stability

After the selection of the target host strain on which genetic transfer will be performed based on its probiotic features, one of the most critical points of genetic engineering applications is to transfer the foreign DNA carrying relevant genes to the host cell to improve the probiotic features. Acceptance of foreign DNA depends on transport across the cell wall and membranes, overcoming host defense systems and active replication inside the host. Entering the cell is possible by changing the composition of the cell wall and porosity of the membrane (chemical transformation or electroporation), injecting DNA (transduction and fusion), or using existing systems in the host cell (natural competence). Often, transformation conditions need to be optimized, even for different strains of the same species. Once foreign DNA enters the host cell, it interacts with host defense systems that try to destroy the invading DNA. The typical examples of these systems are restriction/modification systems, CRISPR/Cas systems, and abortive infection systems. Restriction/modification systems are systems found in almost all bacterial species and thus constitute the biggest obstacle to DNA transformation (Landete, Arqués, & Peirotén, 2014). Three different methods have been developed to overcome this obstacle: (1) using an intermediate host that can be easily transformed with compatible methylation patterns; (2) using a regulated intermediate that expresses the predicted methyltransferases present in the target microorganism; and (3) in vitro incubation with commercially available methyltransferases to match DNA methylation patterns. Using this technique, a similar conversion can be achieved with methylation levels of *Escherichia coli* after the treatment of *Lb. plantarum* with methyltransferases in the intestinal flora. Foreign DNA is transferred into the cell after the host acceptance. It can be stabilized with the help of selectable markers and a compatible origin of replication or by integrating a region into the genome through specific recombination. The first large host sequence plasmid replicons suitable for heterologous gene expression in many LABs and even *E. coli* are pWV01, pSH71, and pAMβ-1. *E. coli-Bifidobacterium* and *E. coli-Bacteroides* shuttle vectors have also been recently produced as food-grade tools to be used in genetic engineering applications (Tauer, Hein, Egger, Heiss, & Grabherr, 2014; Waller, Bober, Nair, & Beisel, 2017).

3.4 Expression systems

To date, different food-grade host/vector combinations have been developed and introduced as homologous or heterologous expression systems. The most typical examples of them are *Lb. plantarum*/pSIP and *Lc. lactis*/NICE (nisin-controlled expression) systems. pSIP expression vectors provide inducible protein expression by means of the natural regulatory systems involved in the bacteriocin production of *Lb. sakei*. However, the most important handicap of these expression vectors is that they have a narrow host range. NICE systems have been developed to provide the expression of different proteins through regulatory systems involved in the production of the antimicrobial peptide called nisin, similar to pSIP. Here the promoter of the *nisA* gene is regulated by a two-component system. These are NisR, a sensor kinase, and NisK, a response regulator. The addition of nisin to the medium promotes transcription from the *nisA* gene promoter via the two-element system. NICE

systems are more preferred expression systems because they contain a much larger host range. Different researchers have developed numerous expression vectors based on the transcriptional or translational fusions of the *nisA* promoter. These vectors are still used successfully in homologous or heterologous gene expression. Since the NICE system is functional not only in *Lc. lactis* but also in many other LAB, it is of great importance in the development of probiotic strains.

The expression of cloned genes can sometimes be insufficient independent of the systems described previously. In many cases, there is a need to adjust expression levels specifically. The most common solution is to adjust the expression of the promoter library by the ribosomal binding site or by modifying the Shine–Dalgarno sequences. With this approach, *Lb. plantarum* and *Bacteroides* have been used to create a promoter library in *Bacteroides thetaiotaomicron* with a varying expression range of about 104 times in different vectors (Mimee, Tucker, Voigt, & Lu, 2015; Horn et al., 2016). Chemical mediated induction systems such as isopropyl β-D-1-thiogalactopyranoside and D-xylose are also successfully used in LAB strains (Horn et al., 2016).

One of the most significant drawbacks of natural induction systems in target host organisms is that they create effects that inhibit natural cell regulation or metabolism. Generally, both natural and unnatural expression systems described earlier are not suitable for general use, as they differ from species to species and even between strain specificity. For these reasons, designing systems that protect their functions when applied to different species is one of the main objectives that must be accomplished before genetic engineering practices. A typical example of such a system is the T7 RNA polymerase system. If the biological system is designed to operate in vivo, it is desirable to design the system to operate only when the organism is at its intended location. For example, heterologous protein expression was induced at high temperature (42°C) in the LAB using Plp-0775, a temperature-sensitive system. However, these systems have not yet robust to respond to a wide range of organisms. Due to the increasing demand in this field, many intensive studies are carried out (O'Sullivan & Klaenhammer, 1993; Kushwaha, Arriga, & Salis, 2015).

3.5 Protein transport

The expression of the gene cloned in a foreign host is not sufficient in many cases. It cannot be taken as an absolute measure of success in genetic engineering applications. For a foreign gene to be expressed effectively in the target bacterial host cell, it is imperative that the gene is designed to operate under the control of the transcription and translation machine of the host cell. For example, if a eukaryotic gene is desired to be expressed in a bacterium, there are some requirements, such as its association with a prokaryotic promoter, its arrangement to include a suitable ribosome binding site, and purification from intervening sequences. Besides, it is also essential to control the cellular localization of the heterologous protein produced if the cloned gene has been transferred into the foreign cell to form an immune response or an environmental sensor naturally present in the cytoplasmic membrane. Proteins can be directed to cellular secretion or the surface of host bacteria using genetic engineering techniques. This technique significantly helps the developed probiotic strains interact directly with host cells or other members of the microbiota. Identification of the N-terminal region of signals recognized by the SecY pathway components in their LABs and directed toward secretion has enabled the addition of regions encoding the secreting signal sequence to the protein of interest. In this way, significant success has been achieved in this area. While the Usp45 secretion signal is widely used in *Lc. lactis*, various secretion signals have been tested in *Lb. plantarum*, and their efficacy has been found to depend on passenger protein domains (Kushwaha, Arriga, & Salis, 2015). The fixing of the protein to the cell surface is accomplished by creating a fusion protein between the cell surface anchor protein and the protein of interest. Anchor proteins are found in various motifs (e.g., transmembrane, lipobox, LysM, and LPxTG), and all of which are used in LAB applications. However, it is still challenging to predict which secretion signal or anchor protein will yield the highest secretion efficiency or desired level of localization because information on the molecular level on these systems is very limited (O'Sullivan & Klaenhammer, 1993). On the other hand, the technique of heterologous binding of proteins produced by recombinant strains to an unmodified bacterial surface is a powerful method to avoid identifying the product in question as GMO. However, in this case, it is necessary to prove that there is no recombinant DNA contamination in the purified product. For this purpose, different heterologous protein display methods such as endolysin Lyb5, SlpB (S layer protein), and DARPins (designed akryin repeat proteins), which are functional in LAB, have been developed (O'Sullivan & Klaenhammer, 1993; Kushwaha, Arriga, & Salis, 2015).

4 Use of CRISPR-Cas systems

CRISPR/Cas systems are adaptive immune systems found in bacteria and archaea. In the CRISPR/Cas9 system, the Cas9 gene encodes an endonuclease enzyme. The repeated spacer array is processed, after transcribing it to a leader, and crRNAs (CRISPR RNAs) are created. These crRNAs are guided endonuclease to cut dsRNA from the specific region. In genome

editing, the efficiency of the system in question is very high, as it is capable of separating edited cells from non-edited ones. The first use of CRISPR/Cas9 systems in bacteria for genome editing was carried out in *Streptococcus pneumonia* and *E. coli*. CRISPR/Cas9 systems have the potential to be used not only in generating site-directed mutations but also for the purpose of cleaning cells that do not recombine.

There are some important points to be considered in the application of this system to probiotic bacteria for genetic engineering purposes. The lack of an effective transformation system to be used for this purpose is a serious problem. Therefore, in non-model organisms, natural CRISPR/Cas systems should be defined first. CRISPR-Cas systems are divided into 11 subtypes under 3 main groups. Each subtype contains its own specific Cas protein and is named based on the model organism. Cas9 is an RNA-guided endonuclease. The second important point is the definition of PAM and seed sequences. These sequences are of great importance in recognition of the target gene and the activity of the system. The third critical point is the realization of strategies to ensure the correct targeting of sgRNA to eliminate erroneous base pairings. Mature CRISPR (cr) RNAs are produced as a result of transcription and processing of CRISPR series. That is, guide RNAs are derived from CRISPRs. CrRNP, a nucleoprotein, is also produced from CRISPR RNA and Cas proteins. This nucleoprotein digests foreign DNA by breaking DNA/RNA hybrids formed in foreign DNA entering the cell (Yadav et al., 2018). CRISPR is guided by its specific guide RNA (sgRNA) to bind to the target DNA sequence. In genetic engineering, vectors are designed for this purpose. Besides CRISPR-Cas sgRNA sequences, codon-specific variants of Cas9, strong promoters of transcription of Cas9, and sgRNA sequences should be found (Sander & Joung, 2014; Sontheimer & Barrangou, 2015).

CRISPR-Cas systems are found in about 40% of bacteria. These systems can also be used to control gene expression without having to change the genome. In total, CRISPR-Cas systems offer a robust set of tools that will benefit the operation of the microbiome and lead to the development of new strategies to replace it. Thanks to this method, it allows the genome to acquire some new properties without antibiotic resistance in probiotic bacteria, or it can be used to control infections by producing probiotic strains specific viruses for intestinal tract pathogens (Posno et al., 1991). Short palindromic repeats (CRISPR-Cas), transcription activator-like effector nuclease, and nuclease (ZEN) with zinc finger motifs are the most common tools for gene regulation in genetic engineering (Ramachandran & Bikard, 2019).

DNA arrangements via CRISPR-Cas systems are catalyzed by the formation of fractures in the target series after complementary guiding series with the target DNA in vitro conditions. At this stage, the broken regions are repaired by recombination between highly homologous regions. In this process, insertions and deletions can be created in the target areas. The first applications of probiotics with CRISPR-Cas systems were successful in *Lb. reuteri* and *S. thermophilus* (Selle, Klaenhammer, & Barrangou, 2015). One of the most robust approaches in the production of commercial probiotic strains is the use of single-chain recombinant DNA fragments (SSDR) to perform chromosomal changes through CRISPR/Cas systems. CRISPR vectors designed accordingly can be transformed into probiotic strains capable of expressing Cas and RecT proteins. The CRISPR plasmid vector containing the CRISPR string, along with Cas9 and tracrRNA, can break the target DNA sequence in the host to allow single-chain DNA to change. Cas9 has been found to be toxic for some probiotic species used in genetic engineering applications. Alternative Cas9 variants were produced and successfully used for the solution of this problem. The best known of these Cas9 variants is ThermoCas9 (Van & Britton, 2014).

Another new application in the use of CRISPR/Cas9 systems in this area is based on editing. In this case, the fusion protein is formed by coupling the cytidine deaminase enzyme with the catalytically impaired Cas9 variant. In this way, base substitutions of cytosine to thymine or guanine to adenine are catalyzed. New techniques developed based on CRISPR/Cas systems have created an unlimited variety of manipulations in probiotics. Now, a series of processes from single nucleotide changes to replacement of gene strings in the desired direction can be achieved quickly and efficiently.

5 Systems biology approaches

Recent advances in life science technologies and system biology have enabled the definition of probiotic–host interactions in a broad perspective. Systems biology is a holistic approach developed to decipher the complexity of biological systems based on the understanding that the work networks that make up all living organisms cannot be expressed by the sum of the parts. In-line with this basic goal, systems develop mathematical and computational models by processing data from genomic, transcriptomic, proteomic, metabolomic, and energetics analysis. Today, in vivo and in vitro studies combined with computer-based methods are widely used in the development of bioactive properties of probiotics that have an impact on human health. There are many microorganisms developed by in silico metabolic engineering applications and presented for industrial, medical, or agricultural purposes (Singh & Shukla, 2011; Van & Britton, 2014; Singh & Shukla, 2015; Singh, Joseph, Goyal, Grover, & Shukla, 2016).

Many in silico tools that assist in modeling cellular metabolic networks, metabolic pathways, and improving cellular properties in this direction have already been used for scientific and industrial purposes. Metabolic trace path analysis

(MPA), flow balance analysis (FBA), and metabolic flow analysis (MFA) are the highest level of in silico metabolic engineering programs. MPA is one of the most preferred methods by bioengineers in understanding the phenotypes emphasized in the producer cells. MFA, on the other hand, provides very reliable data in determining the effects of chemicals or drugs on cellular metabolism. Finally, FBA is the approach that expresses the flow of metabolites in a metabolic network as a flow balance with a mathematical approach. In this sense, MPA, MFA, and FBA provide a unique aid in the production, regulation, and diversification of bioactive products from probiotics (Ho & Mittal, 1996; Van Baarlen et al., 2010). On the other hand, advancements in quantitative structure–activity relationships (QSAR) and its atom-based models, 3D-QSAR3D technologies, have become powerful tools to define the activity of probiotic metabolites in the host organism at molecular levels. For example, QSAR atom-based methods have provided great benefits in defining the antihypertensive effects of probiotic metabolites (Singh et al., 2016).

The rapid development of gene sequence analysis techniques has resulted in the rapid completion of the total genome sequencing of probiotic bacteria. Computer-based combinations and analysis of these data with transcriptomic, proteomic, and metabolomic data brought the realization of metabolic-level models. Genome-scale metabolic models (GEMs) have been identified and introduced for many organisms, particularly *Lactobacillus*, *Enterococcus*, *Bacillus*, *Bifidobacterium*, and *E. coli*. These models are essential tools for identifying and producing different bioactive metabolites in probiotic strains.

6 Biosafety

Probiotics have been the focus of genetic engineering studies, especially in designing as effective agents against foodborne pathogens. However, the most important disadvantage of probiotic strains designed for these or similar purposes is that they are considered GMO. Therefore, these applications contain serious limitations. Especially consumers' reactions to GMO products covering ethical and health issues constitute the main source of these limitations. In addition, the release of GMOs to nature has the potential to disrupt natural balance due to the competition they will create with wild type species. The process, called self-cloning, is excluded from the definition of genetically modified microorganisms (Douglas, Goh, & Klaenhammer, 2011). Since LAB and *Bifidobacteria* are GRAS microorganisms, the practices mentioned earlier using these organisms in some countries are considered non-GMO and food-grade (Lu & Kong, 2013).

It is a nonscientific prediction that to say the use of food-grade cloning systems consisting of DNA from the homologous host or GRAS organisms, free of antibiotic selection criteria, is entirely harmless for consumer health or the environment. The process of gaining new features to the host organisms by transferring and rearranging DNA to the recipient strain does not only produce foreseen results. However, undesirable effects that may be the result of this change may also occur in recombinants. Various food-level strategies are tried to be developed to minimize these unexpected effects. Undoubtedly, the rationale for the development of LAB and cloning vectors derived from cryptic plasmids of LAB is due to the need to access new foodborne genetic engineering tools. Here, the vector generation strategy is to use replicons of small cryptic plasmids and selectable food-grade markers such as lactose fermentation, nisin resistance, or nisin immunity. However, the ability to perform a limited gene cloning with these cryptic vector systems is the main limiting factor in the industrial use of these vectors (Shareck et al., 2004).

On the other hand, until now, the modification of only a few nucleotides has been accomplished using the CRISPR system in LAB (van Pijkeren & Britton, 2012; van Pijkeren, Neoh, Sirias, Findley, & Britton, 2012; Oh & van Pijkeren, 2014). For this technique to be used on an industrial scale, there is a need to develop laboratory applications that will regulate much longer gene sequences in LAB and *Bifidobacteria* (Stefanovic, Fitzgerald, & McAuliffe, 2017). It is also common to see that organisms modified by the CRISPR system are also not considered GMOs. However, whether they are applications with food-grade vectors or CRISPR applications for different purposes, they are challenging in vitro applications, and the most important point that should not be overlooked is the fact that these applications will affect not only the interfering DNA region but the entire genome.

Many countries still do not have legal regulations for the biosafety and evaluation criteria of recombinant probiotics. Because all genome functions of organisms studied in such a situation that has been subjected to genetic engineering techniques are not resolved, it is impossible to say that the results are completely safe for humans, animals, and the environment, regardless of which in vitro technique is applied. On the other hand, the fact that the genetic modifications defined within the scope of this section can still be realized on a small scale is the main factor that limits their industrial use. Therefore, it is only possible to respond to industrial needs with the development of techniques that allow genetic interventions to be carried out on a larger scale. This will strengthen the interactions between newly added genes and genes on the genome, and the possibility of unpredictable genetic-metabolic results. Although this is a very clear scientific fact, excluding the probiotic strains obtained by the genetic engineering practices outlined in this section out of the GMO definition is

a misidentification created with much haste and market anxiety. In all countries that have signed the Cartagena biosecurity contract, GMO definition and safety limitations are determined by this contract. While it is often possible to stretch the definitions and overcome safety regulations in this way, in many GMO applications, the genetic metabolic defects of the product appear at the market stage or withdraw from the market due to consumer rejection.

All organisms developed with genetic engineering techniques are in the GMO definition, and their production and placing on the market are subject to international contracts. These contracts are above national laws when these products create biosafety risks or in the presence of such a possibility. Given all these facts and the current state of natural sciences, it would not be prophetic to say that there is still a long way for science and humanity to reach the market stage of GMO probiotics. All steps to be taken toward the delivery of these products to the consumer before full security is achieved are serious risks.

7 Conclusion

Probiotics, in their most general definition, are living microorganism cultures that provide a health benefit in the host organism when administered in appropriate quantities. In particular, *Lactobacillus* and *Bifidobacterium* spp. are of great interest as probiotics as they can be used within a wide range of adjuvants or prophylaxis approaches such as neuropsychiatric disorders, cancer, intestinal syndrome, and urinary tract infections (Mays & Nair, 2018). Probiotic preparations are also included in many animal husbandry practices, including chickens, cows, pigs, and fish, to increase productivity and prevent disease. On the other hand, the most widely used field of probiotics today is the food industry (Syngai, Gopi, & Bharali, 2016). The probiotic market is estimated to reach $46.55 billion by 2020. Today, the production and commercialization of probiotics are often not properly regulated on the basis of their efficacy and quality control. For this reason, in the growing global probiotic market, consumers have serious problems about how to distinguish between high- and poor-quality products. The minimum condition in probiotic products should be to define the benefits of living microorganisms used on consumer health in a way that will not cause any doubt. However, it has been proven that some probiotic preparations do not meet the minimum requirements, and they are products produced to mislead the consumer. Although numerous scientific literature data indicate the beneficial effects of probiotics, the most important points for limiting the reliability of health claims are the lack of molecular and mechanical information about the in vivo mechanism of action of probiotics and very low reproducibility of trials, as well as strong individual responses and strain specificity of host organisms (Grohmann, Muth, & Espinosa, 2003; Yoshida & Sato, 2009; Hale et al., 2012; Kaswurm, Nguyen, Maischberger, Kulbe, & Michlmayr, 2013; Syngai et al., 2016; Lebeer, Bron, & Marco, 2018). The quality and reliability of probiotics depend on a detailed description of all biological features of probiotic strains and, accordingly, host/probiotic microorganism interactions. This requires a holistic definition of data from the use of other omic technologies combined with genomic approaches, based on a systems biology approach.

Genetic engineering applications to be carried out in probiotic strains are possible to develop probiotics with superior characteristics. Such GMO probiotics have many potential applications. The main areas of application are medical therapy, food industry, and the production of different metabolites. The foremost problem of GMO probiotic application areas is their use for food purposes. A series of vector systems have been developed to enable probiotic strains, designed with genetic engineering techniques, to be used in food production. These vectors are food-level cloning and expression vectors.

On the other hand, the use of GRAS-level well-defined strains as hosts is another criterion that increases their reliability. In addition to these, the increased possibilities of using CRISPR/Cas9 systems in the genetic modification of probiotic strains paves the way for genetic engineering applications to be carried out in this field. Early steps to take on GMO probiotics due to market anxiety can lead to unrecoverable negative results, as these are preparations used at regular and high doses. The fact that genetic intervention is irreversible imposes scientific and ethical responsibilities on practitioners. In this context, making legal regulations is the only guarantee of developing probiotic applications without moving away from a scientific basis.

References

Abubucker, S., Segata, N., Goll, J., Schubert, A., Izard, J., & Cantarel, B. (2012). Metabolic reconstruction for metagenomic data and its application to the human microbiome. *PLoS Computational Biology*, *8*, e1002358.

Aune, T. E. V., & Aachmann, F. L. (2010). Methodologies to increase the transformation efficiencies and the range of bacteria that can be transformed. *Applied Microbiology and Biotechnology*, *85*, 1301–1313.

Biswas, I., Gruss, A., & Ehrlich, S. D. (1993). High-efficiency gene inactivation and replacement system for Gram-positive bacteria. *Journal of Bacteriology*, *175*, 3628–3635.

Byndloss, M. X., Pernitzsch, S. R., & Bäumler, A. J. (2018). Healthy hosts rule within: Ecological forces shaping the gut microbiota. *Mucosal Immunology*, *11*, 1299–1305.

Cani, P. D. (2018). Human gut microbiome: Hopes, threats and promises. *Gut*, *67*, 1716–1725.

Douglas, G. L., Goh, Y. J., & Klaenhammer, T. R. (2011). Integrative food grade expression system for lactic acid bacteria. In J. A. Williams (Ed.), *Strain engineering: Methods and protocols, methods in molecular biology* (pp. 373–387). (Vol. 765). New Jersey, USA: Springer Science+Business Media.

El Demerdash, H. A. M., Heller, K. J., & Geis, A. (2003). Application of the *shsp* gene, encoding a small heat shock protein, as a food-grade selection marker for lactic acid bacteria. *Applied and Environmental Microbiology*, *69*, 4408–4412.

Espinosa, M., Cohen, S., & Couturier, M. (2000). Plasmid replication and copy number control. In C. M. Thomas (Ed.), *The horizontal Gene Pool: Bacterial plasmids and gene spread* (pp. 1–47). Amsterdam: Harwood Academic Publishers.

Fukoi, H. (2019). Role of gut dysbiosis in liver diseases: What have we learned so far? *Disease*, *7*, 1–42.

Gomes, J. M. G., Costa, J. A., & Alfenas, R. C. G. (2017). Metabolic endotoxemia and diabetes mellitus: A systematic review. *Metabolism*, *68*, 133–144.

Gosalbes, M. J., Esteban, C. D., Galan, J. L., & Perez-Martinez, G. (2000). Integrative food-grade expression system based on the lactose regulon of *Lactobacillus casei*. *Applied and Environmental Microbiology*, *66*, 4822–4828.

Gosalbes, M., Durban, A., Pignatelli, M., Abellan, J., Jimenez-Hernandez, N., & Perez-Cobas, A. (2011). Metatranscriptomic approach to analyze the functional human gut microbiota. *PLoS One*, *6*, e17447.

Grohmann, E., Muth, G., & Espinosa, M. (2003). Conjugative plasmid transferrin gram-positive bacteria. *Microbiology and Molecular Biology Reviews*, *67*, 277–301.

Hale, C. R., Majumdar, S., Elmore, J., Pfister, N., Compton, M., Olson, S., et al. (2012). Essential features and rational design of CRISPR RNAs that function with the Cas RAMP module complex to cleave RNAs. *Molecular Cell*, *45*, 292–302.

Heap, J. T., Ehsaan, M., & Cooksley, C. M. (2012). Integration of DNA into bacterial chromosomes from plasmids without a counter-selection marker. *Nucleic Acids Research*, *40*(8), e59.

Hemarajata, P., & Versalovic, J. (2013). Effects of probiotics on gut microbiota: Mechanisms of intestinal immunomodulation and neuromodulation. *Therapeutic Advances in Gastroenterology*, *6*(1), 39–51.

Ho, S. Y., & Mittal, G. S. (1996). Electroporation of cell membranes: A review. *Critical Reviews in Biotechnology*, *16*, 349–362.

Horn, N., Carvalho, A. L., Overweg, K., Wegmann, U., Carding, S. R., & Stentz, R. (2016). A novel tightly regulated gene expression system for the human intestinal symbiont *Bacteroides thetaiotaomicron*. *Frontiers in Microbiology*, *7*, 1080–1086.

Huttenhower, C., Gevers, D., Knight, R., Abubucker, S., Badger, J. H., Chinwalla, A. T., et al. (2012). Structure, function and diversity of the healthy human microbiome. *Nature*, *486*, 207–214.

Jie, Z., Xia, H., Zhong, S. L., Feng, Q., Li, S., Liang, S., et al. (2017). The gut microbiome in atherosclerotic cardiovascular disease. *Nature Communication*, *8*(1), 845–849.

Kaswurm, V., Nguyen, T. T., Maischberger, T., Kulbe, K. D., & Michlmayr, H. (2013). Evaluation of the food grade expression systems NICE and pSIP for the production of 2,5-diketo-D-gluconic acid reductase from *Corynebacterium glutamicum*. *AMB Express*, *3*, 7–16.

Kushwaha, M., Arriga, G., & Salis, H. M. (2015). A portable expression resource for engineering cross-species genetic circuits and pathways. *Nature Communications*, *6*, 1–11, 7832.

Lahteinen, T., Malinen, E., Koort, J. M., Mertaniemi-Hannus, U., Hankimo, T., Karikoski, N., et al. (2010). Probiotic properties of *Lactobacillus* isolates originating from porcine intestine and feces. *Anaerobe*, *16*, 293–300.

Landete, J. M., Arqués, J. L., & Peirotén, A. (2014). An improved method for the electrotransformation of lactic acid bacteria: A comparative survey. *Journal of Microbiological Methods*, *105*, 130–133.

Landete, J. M. (2016). Effector molecules and regulatory proteins: Applications. *Trends in Biotechnology*, *34*(10), 777–780.

Landete, J. M. (2017). A review of food-grade cloning vectors in lactic acid bacteria: From the laboratory to their application. *Critical Review in Biotechnology*, *37*, 296–308.

Larsson, E., Tremaroli, V., Lee, Y., Koren, O., Nookaew, I., & Fricker, A. (2012). Analysis of gut microbial regulation of host gene expression along the length of the gut and regulation of gut microbial ecology through Myd88. *Gut*, *61*, 1124–1131.

Lebeer, S., Bron, P. A., & Marco, M. L. (2018). Identification of probiotic effector molecules: Present state and future perspectives. *Current Opinion in Biotechnology*, *49*, 217–223.

Lederberg, J., & McCray, A. (2001). 'Ome sweet' omics: A genealogical treasury of words. *Scientist*, *15*, 8–13.

Lu, W., & Kong, J. (2013). Construction and application of a food-grade expression system for *Lactococcus lactis*. *Molecular Biotechnology*, *54*, 170–176.

Mays, Z. J., & Nair, N. U. (2018). Synthetic biology in probiotic lactic acid bacteria: At the frontier of living therapeutics. *Current Opinion in Biotechnology*, *53*, 224–231.

Methe, B. A., Nelson, K. E., Pop, M., Creasy, H. H., Giglio, M. G., Huttenhower, C., et al. (2012). A framework for human microbiome research. *Nature*, *486*(7402), 215–221.

Mimee, M., Tucker, A. C., Voigt, C. A., & Lu, T. K. (2015). Programming a human commensal bacterium, *Bacteroides thetaiotaomicron*, to sense and respond to stimuli in the murine gut microbiota. *Cell Systems*, *1*, 62–71.

Mu, C., Yang, Y., & Zhu, W. (2016). Gut microbiota: The brain peacekeeper. *Frontiers in Microbiology*, *7*, 1–11, 345.

Nishino, K., Nishid, A., Inoue, R., Kawada, Y., Ohno, M., Sakai, S., et al. (2018). Analysis of endoscopic brush samples identified mucosa-associated dysbiosis in inflammatory bowel disease. *Journal of Gastroenterology*, *53*(1), 106–113.

Nobaek, S., Johansson, M., Molin, G., Ahrne, S., & Jeppsson, B. (2000). Alteration of intestinal microflora is associated with reduction in abdominal bloating and pain in patients with irritable bowel syndrome. *American Journal of Gastroenterology*, *95*, 1231–1238.

O'Sullivan, D. J., & Klaenhammer, T. R. (1993). High- and low-copy-number *Lactococcus* shuttle cloning vectors with features for clone screening. *Gene*, *137*, 227–231.

Oh, J. H., & van Pijkeren, J. P. (2014). CRISPR-Cas9-assisted recombineering in *Lactobacillus reuteri*. *Nucleic Acids Research*, *42*(17), e131.

Peterbauer, C., & Haltrich, D. (2011). Food-grade gene expression in lactic acid bacteria. *Biotechnology Journal*, *6*, 1–15.

Pflughoeft, K., & Versalovic, J. (2012). Human microbiome in health and disease. *Annual Review of Pathology*, *7*, 99–122.

Posno, M., Leer, R. J., & van Luijk, N. (1991). Incompatibility of *Lactobacillus* vectors with replicons derived from small cryptic *Lactobacillus* plasmids and segregational instability of the introduced vectors. *Applied and Environmental Microbiology*, *57*, 1822–1828.

Radilla-Vázquez, R. B., Parra-Rojas, I., & Martínez-Hernández, N. E. (2016). Gut microbiota and metabolic endotoxemia in young obese Mexican subjects. *Obesity Facts*, *9*, 1–11.

Ramachandran, G., & Bikard, D. (2019). Editing the microbiome the CRISPR way. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *13*, 374–381.

Sander, J. D., & Joung, J. K. (2014). CRISPR-Cas systems for editing, regulating and targeting genomes. *Nature Biotechnology*, *32*, 347–355.

Selle, K., Klaenhammer, T. R., & Barrangou, R. (2015). CRISPR-based screening of genomic island excision events in bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, *112*, 8076–8081.

Shareck, J., Choi, Y., Lee, B., & Miguez, C. B. (2004). Cloning vectors based on cryptic plasmids isolated from lactic acid bacteria: Their characteristics and potential applications in biotechnology. *Critical Review in Biotechnology*, *24*, 155–208.

Singh, P. K., & Shukla, P. (2011). Molecular modeling and docking of microbial inulinases towards perceptive enzyme-substrate interactions. *Indian Journal of Microbiology*, *52*, 373–380.

Singh, P. K., & Shukla, P. (2015). Systems biology as an approach for deciphering microbial interactions. *Briefings in Functional Genomics*, *14*, 166–168.

Singh, P. K., Joseph, J., Goyal, S., Grover, A., & Shukla, P. (2016). Functional analysis of the binding model of microbial inulinases using docking and molecular dynamics simulation. *Journal of Molecular Modelling*, *22*, 1–7.

Sommer, F., Anderson, J. M., Bharti, R., Raes, J., & Rosenstiel, P. (2017). The resilience of the intestinal microbiota influences health and disease. *Nature Reviews Microbiology*, *15*, 630–638.

Song, L., Cui, H., Tang, L., Qiao, X., Liu, M., Jiang, Y., et al. (2014). Construction of *upp* deletion mutant strains of *Lactobacillus casei* and *Lactococcus lactis* based on counter selective system using temperature-sensitive plasmid. *Journal of Microbiological Methods*, *102*, 37–44.

Sontheimer, E., & Barrangou, R. (2015). The bacterial origins of the CRISPR genome editing revolution. *Human Gene Therapy*, *26*, 413–424.

Stefanovic, E., Fitzgerald, G., & McAuliffe, O. (2017). Advances in the genomics and metabolomics of dairy lactobacilli: A review. *Food Microbiology*, *61*, 33–49.

Syngai, G. G., Gopi, R., & Bharali, R. (2016). Probiotics—The versatile functional food ingredients. *Journal of Food Science and Technology*, *53*, 921–933.

Takala, T. M., Saris, P. E. J., & Tynkkynen, S. S. H. (2005). Food-grade host/vector expression system for *Lactobacillus casei* based on complementation of plasmid-associated phospho-b-galactosidase gene *lacG*. *Applied Microbiology and Biotechnology*, *60*, 564–570.

Tauer, C., Hein, I. S., Egger, E., Heiss, S., & Grabherr, R. (2014). Tuning constitutive recombinant gene expression in *Lactobacillus plantarum*. *Microbial Cell Factories*, *13*, 150–158.

Van, P. J. P., & Britton, R. A. (2014). Precision genome engineering in lactic acid bacteria. *Microbial Cell Factories*, *13*, 1–10.

Van Baarlen, P., Troost, F., Van Der Meer, C., Hooiveld, G., Boekschoten, M., & Brummer, R. (2010). Human mucosal in vivo transcriptome responses to three lactobacilli indicate how probiotics may modulate human cellular pathways. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(Suppl. 1), 4562–4569.

van Pijkeren, J. P., & Britton, R. A. (2012). High efficiency recombineering in lactic acid bacteria. *Nucleic Acids Research*, *40*(10), e76.

van Pijkeren, J. P., Neoh, K. M., Sirias, D., Findley, A. S., & Britton, R. A. (2012). Exploring optimization parameters to increase ssDNA recombineering in *Lactococcus lactis* and *Lactobacillus reuteri*. *Bioengineered*, *3*(4), 209–217.

Ventura, M., Turroni, F., & van Sinderen, D. (2012). Probiogenomics as a tool to obtain genetic insights into adaptation of probiotic bacteria to the human gut. *Bioengineered Bugs*, *3*, 73–79.

Waller, M. C., Bober, J. R., Nair, N. U., & Beisel, C. L. (2017). Toward a genetic tool development pipeline for host-associated bacteria. *Current Opinion in Microbiology*, *38*, 156–162.

Yadav, R., Kumar, V., Baveja, M., & Shukla, P. (2018). Gene editing and genetic engineering approaches for advanced probiotics: A review. *Critical Reviews in Food Science and Nutrition*, *10*, 1735–1746.

Yoshida, N., & Sato, M. (2009). Plasmid uptake by bacteria: A comparison of methods and efficiencies. *Applied Microbiology and Biotechnology*, *83*, 791–798.

Chapter 7

Biosynthetic Gene Cluster Analysis in *Lactobacillus* Species Using antiSMASH

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1 Introduction

It is scientifically stated that certain species of microorganisms can make us unwell and can even be noxious, in contrast to them the live bacteria and yeasts that are good for humans, especially to the digestive system, are referred to as probiotics. We often think that these microorganisms can cause diseases. But the body is already filled with bacteria, both good and bad. The so-called "good" or "helpful" bacteria are called probiotics as they help in keeping the gut healthy.

Several multidrug-resistant bacteria that have been causing important healthcare-associated infections and dangerous serotypes for the human lives have been causing serious emerging food poisonings due to production of enterotoxins. Some of these medically important bacteria include methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, extended-spectrum beta-lactamase-producing Enterobacteriaceae, multidrug-resistant *Pseudomonas aeruginosa*, multidrug-resistant *Mycobacterium tuberculosis*, and Enterohemorrhagic *Escherichia coli* (Fijan, 2014; Gruber et al., 2013; Fijan & Šostar-Turk, 2012).

The beneficial reports on the effects of probiotic consumption which includes the improvement of intestinal health, amelioration of symptoms of lactose intolerance, and reduction of the risk of various other diseases, and several well-characterized strains of lactobacilli and bifidobacteria are available for human use. Lactose intolerance is a genetically determined beta-galactosidase deficiency resulting in the inability to hydrolyze lactose into the monosaccharides glucose and galactose. Upon reaching the large bowel, the undigested lactose is degraded by bacterial enzymes leading to osmotic diarrhoea. Acquired, usually reversible, causes of beta-galactosidase deficiency include pelvic radiotherapy that damages the mucosa, as well as infection with rotavirus which infects lactase-producing cells, and short bowel syndrome. Lactose-intolerant individuals develop diarrhoea, abdominal discomfort, and flatulence after consumption of milk or milk products. Although conventional yoghurt preparations, using *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus,* are even more effective in this direction, partly due to of higher beta-galactosidase activity, improvement of metabolism in lactose is a claimed health benefit attributed to probiotics and seems to involve certain strains more than others and in specific concentrations. Therefore and as certain individuals have responded positively to probiotic supplementation, clinicians should consider it as a therapeutic alternative (Kechagia et al., 2013).

Taking into consideration their definition, the quantity of microbial species that may exert probiotic properties is impressive. As far as nutrition is concerned, only the strains classified as lactic acid bacteria are of significance and among them the ones with the most important properties in an applied context are those belonging to the genera *Lactococcus* and *Bifidobacterium* (Holzapfel, Haberer, Geisen, Björkroth, & Schillinger, 2001).

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00007-1
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2 In vitro and in vivo studies on beneficial effects of probiotics

2.1 Bowel diseases and the immune system

Ulcerative colitis and Crohn's diseases are types of bowel diseases that have been linked to the gut's microbial genetic predisposition and environment. The imbalance between the intestinal immunity and the microbiome may cause these bowel diseases. Enteric bacteria may change the equilibrium of proinflammatory and antiinflammatory cytokine level of the intestine that becomes the predisposing factor for intestinal disorders. Proinflammatory cytokines are produced by Th1 cells and antiinflammatory cytokines are secreted by Th2 cells which are important in maintaining the homeostasis of the immune system inside the intestinal barrier. Probiotic organisms are increasingly known for its ability to prevent or treat the intestinal disorders and improve the immune system in both in vitro and animal models (Lye, Balakrishnan, Thiagarajah, Mohd Ismail, & Ooi., 2016).

2.2 Dermal health

Probiotics have been proved to show some new benefits for skin health. Even recent studies suggested that probiotics could improve wound, atopic eczema, and scar healing and help in skin rejuvenation (Lye et al., 2016).

2.3 Dental caries

It is a bacterially mediated process, dental caries, that is characterized by the acid demineralization of tooth enamel. In the process of preventing dental caries, probiotics need to adhere to the dental surfaces and antagonize the cariogenic species like mutants streptococci and lactobacilli. Probiotics that are incorporated into the dairy products such as cheese could neutralize the acidic conditions in the mouth and prevent demineralization of the tooth enamel (Lye et al., 2016).

3 Modulation of gut–brain axis by probiotics

In a human body the gastrointestinal tract (GIT) is the most highly colonized organ by various species of bacteria such as *Bacteroidetes*, *Firmicutes*, and *Actinobacteria*. The human GIT is inhabited with 10^{13}–10^{14} microorganisms, which is almost tenfold greater than the human cell number and carries about 150 times more genes than that of the human genome. On the other hand, gut–brain axis is the bidirectional interactions between the GIT and the brain. The scaffolding of the gut–brain axis consists of the central nervous system (CNS), the enteric nervous system, the sympathetic and parasympathetic arms of the autonomic nervous system, the neuroendocrine and neuroimmune systems, and also the gut microbiota. A complex reflex network is then formed to facilitate the signaling along the axis, with afferent fiber projections to integrative CNS structures and efferent fiber projections that project to the smooth muscle in the intestinal wall. Through this bidirectional communication network, brain signals can cause a negative effect to the motor, sensory, and secretory functions of the GIT and contrarily, the GIT signals can affect the brain functions. A study suggests that probiotics can regulate in the proper modulation of gut–brain axis (Lye et al., 2016).

Gene cluster that codes for nonribosomal peptide synthetases (NRPSs) and a polyketide synthase (PKS) genes in lactic acid bacteria particularly in *Lactobacillus* is very significant to reveal the several secondary metabolites productions in fermented probiotic food and probiotic supplements as drug for both human and animal consumption. However, there is no detailed report on gene clusters in probiotic microorganism genome using AntiSMASH. Keeping the previous points in mind, we are intended to find out the role of secondary metabolites, including terpene, lanthipeptide, LAP, T3PKS, and NRPS. *Lactobacillus* species is evident of its natural importance to the probiotic microbes in their genome, chromosome, plasmid, and whole-genome shotgun (WGS) sequences.

4 Materials and methods

4.1 Selection of genes from GenBank

GenBank, built by NCBI, is a comprehensive open access database of nucleotide sequences and supporting bibliographical and biological annotation. GenBank is primarily built from the submission of sequence data from authors and individual laboratories dealing in large-scale sequencing projects for WGS sequencing as well as environmental sampling (Benson, Karsch-Mizrachi, Lipman, Ostell, & Wheeler, 2008). The *Lactobacillus* sp. genomes used in this study were the ones that

are chosen and commonly used as probiotics. An information table about all the genomes available was created. The collected genomes were of many types like complete genomes, chromosome, plasmid, and WGS sequences.

4.2 Scrutiny of dataset

Sequences with status complete or chromosome in their genome annotation reports and for which information about their isolation source is available were selected. Metadata about the isolation source, location of isolation, genome size, and incidence of plasmids were collected from the GenBank files of the sequences as well as from review of literature. Nucleotide sequences in FASTA format of the whole genomes were collected from GenBank DNA database for all *Lactobacillus* strains justifiably selected.

5 Secondary metabolite clusters identification using antiSMASH

antiSMASH is a software pipeline developed in 2011 for automatic secondary metabolite gene cluster identification, annotation, and analysis. The advantages of antiSMASH are that it is comprehensive, rapid, and user-friendly. Previous in silico methods like ClustScan (Starcevic et al., 2008) and SBSPKS toolbox (Anand et al., 2010) are limited to type 1 PKS and NRPS analysis. antiSMASH is more comprehensive and covers more cluster classes like lantipeptides, bacteriocins, and terpenes. Secondary metabolite gene cluster analysis of the selected genomes was carried out using antiSMASH 4.0 (Blin et al., 2017) using the GenBank accession number as input and default parameters and ClusterFinder option off.

6 Phylogenetic analysis of genes

The PKS, nonribosomal peptide synthase genes, and other synthase genes used for secondary metabolite production are vital for proper synthesis to take place. Some metabolites like bacteriocin, terpene, lanthipeptide, and LAP were found with 100% similarity in many of the selected genomes by antiSMASH and some were also derived from NCBI (in FASTA format). The amino acid sequences of these core enzymes were collected and subjected to multiple sequence alignment using Clustal Ω in MEGA-X. Later sequences were constructed as phylogenetic trees with the help of MEGA-X for further understandings and analysis.

7 pH concentration

After the phylogenetic analysis, the top five potent *Lactobacillus* sp. were selected and their optimum pH value at which they are considered to be at their maximum efficiency was taken and recorded to finalize the superlative and safe probiotic supplement compatible for human use.

8 Result and discussion

8.1 Selection of genes from GenBank

A total of 27 diverse species of *Lactobacillus* sp. were taken into consideration and their genomes were derived from NCBI(*L. johnsonii*, *L. crispatus*, *L. acidophilus*, *L. helveticus*, *L. reuteri*, *L. plantarum*, *L. fermentum*, *L. casei*, *L. rhamnosus*, *L. paracasei*, *L. brevis*, *L. salivarius*, *L. sakei*, *L. acidipiscis*, *L. ingluviei*, *L. agilis*, *L. gallinarum*, *L. perolens*, *L. collinoides*, *L. ruminis*, *L. coryniformis*, *L. amylovorus*, *L. buchneri*, *L. paraplantarum*, *L. jensenii*, *L. delbrueckii*, and *L. gasseri*) among which, only 22 species genes showed results in antiSMASH. Simultaneously, gene coding for secondary metabolite like PKS and gene coding for lactase were also retrieved from NCBI.

8.2 Scrutiny of dataset

The dataset of the remaining 22 genomes were used for further screening. The isolation source and isolation of these genomes were determined from their GenBank entries and also from review of literature. A few genomes have been directly submitted to NCBI and are unpublished. The information about the isolation source was only found for 21 for lactase concentration and 7 for PKS *Lactobacillus* strains and these strains form the final dataset. The details of the strains like genome size, and GenBank accession IDs are given in Table 7.1.

TABLE 7.1 *Lactobacillus* species with GenBank accession IDs and genome size.

S. no.	*Lactobacillus* sp.	GenBank accession IDs	Genome size (bp)
1	*L. johnsonii*	NC_005362.1	1,992,676
2	*L. crispatus*	NC_014106.1	20,431,618
3	*L. acidophilus*	NC_006814.3	1,993,560
4	*L. helveticus*	NZ_CP012381.1	2,160,583
5	*L. reuteri*	NC_009513.1	1,999,618
6	*L. plantarum*	NC_004567.2	3,308,273
7	*L. fermentum*	NC_010610.1	2,098,685
8	*L. casei*	NZ_AP012544.1	2,924,929
9	*L. rhamnosus*	NC_013198.1	3,010,111
10	*L. paracasei*	NC_008526.1	2,895,264
11	*L. brevis*	NC_008497.1	2,291,220
12	*L. salivarius*	NC_007929.1	1,827,111
13	*L. sakei*	NZ_CP020459.1	1,945,884
14	*L. acidipiscis*	NZ_LT630287.1	2,607,423
15	*L. ingluviei*	NZ_HE997173.1	1,181,616
16	*L. agilis*	NZ_CP016766.1	2,187,209
17	*L. gallinarum*	NZ_CP012890.1	2,317,164
18	*L. perolens*	NZ_AZEC01000001.1	544,927
19	*L. collinoides*	NZ_AYYR01000023	91,765
20	*L. ruminis*	NC_015975.1	2,066,652
21	*L. coryniformis*	NZ_AZCN01000001.1	91,277
22	*L. amylovorus*	NC_015214.1	2,078,001
23	*L. buchneri*	NC_018610.1	2,500,564
24	*L. paraplantarum*	NZ_CP032744.1	3,133,857
25	*L. jensenii*	NZ_CP018809.1	1,672,949
26	*Lactobacillus delbrueckii*	NC_008054.1	1,864,998
27	*L. gasseri*	NC_008530.1	1,894,360

9 Secondary metabolite clusters identification using antiSMASH

The antiSMASH analysis of the previously selected genomes was run using default parameters and ClusterFinder option off. The types of clusters identified include bacteriocin, lantipeptide, NRPS, PKS, terpene, and LAP that are represented in Table 7.2. The hybrid clusters formed are more than the total clusters detected; the maximum among all types of clusters detected. This is because antiSMASH predicts extra genes on both sides of a putative cluster where additional genes from other clusters may be present. The PKS and NRPS clusters are of significant importance as most antibiotics are polyketides and nonribosomal peptides. PKSs are one of the most profound biosynthetic factories for producing polyketides with varying structures and biological activities (Cai & Zhang, 2018). PKS activates the aryl hydrocarbon receptor, a ligand-activated transcription factor that plays an important role in mucosal immune system, by producing tryptophan catabolites (Özçam et al., 2018). The NRPSs are modular enzymes that catalyze synthesis of important peptide products from a variety of standard and nonproteinogenic amino acid substrates. Within a single module are multiple catalytic domains that are responsible for the incorporation of a single residue (Miller & Gulick, 2016).

TABLE 7.2 Biosynthetic gene cluster analysis in *Lactobacillus* sp. using antiSMASH.

Biosynthetic gene cluster analysis in lab using anti smash							
***Lactobacillus* sp.**							
	Bacteriocin	**T3PKS**	**NRPS**	**Terpene**	**Lanthipeptide**	**LAP**	**References**
L. johnsonii	1	0	0	0	0	0	NC_005362.1
L. crispatus	1	**1**[a]	0	0	0	0	NC_014106.1
L. acidophilus	1	0	0	0	0	0	NC_006814.3
L. helveticus	0	0	0	0	0	0	NZ_CP012381.1
L. reuteri	0	1	0	0	0	0	NC_009513.1
L. plantarum	1	1	1	1	0	0	NC_004567.2
L. fermentum	0	0	0	1	0	0	NC_010610.1
L. casei	2	**1**[a]	0	0	0	0	NZ_AP012544.1
L. rhamnosus	1	1	0	0	0	0	NC_013198.1
L. paracasei	2	**1**[a]	0	0	0	0	NC_008526.1
L. brevis	0	1	0	0	1	0	NC_008497.1
L. salivarius	1	1	0	0	0	0	NC_007929.1 NC_007930.1
L. sakei	0	1	0	0	0	1	NZ_CP020459.1 NZ_CP020460.1
L. acidipiscis	0	0	0	0	0	0	NZ_LT630287.1
L. ingluviei	1	1	0	0	0	0	NZ_HE997174.1 NZ_HE997173.1
L. agilis	0	1	0	0	0	0	NZ_CP016766.1
L. gallinarum	0	0	0	0	0	1	NZ_CP012890.1
L. perolens	0	0	0	0	0	0	NZ_AZEC01000001.1
L. collinoides	0	1	0	0	0	0	NZ_AYYR01000023
L. ruminis	1	1	0	0	0	0	NC_015975.1
L. coryniformis	0	**1**[a]	0	0	0	0	NZ_AZCN01000001.1
L. amylovorus	1	0	0	0	0	0	NC_015214.1
L. buchneri	0	1	0	0	0	0	NC_018610.1
L. paraplantarum	1	1	0	1	0	0	NZ_CP032744.1
L. jensenii	0	0	1	0	0	0	NZ_CP018809.1
Lactobacillus delbrueckii	0	0	0	0	1	0	NC_008054.1
L. gasseri	0	0	0	0	0	0	NC_008530.1

[a]*Genes directly derived from NCBI.*

10 Phylogenetic analysis of genes

Since we know that a phylogenetic tree of relationships is the central underpinning of research in many areas of biology. Comparisons of plant species or gene sequences in a phylogenetic analysis context can provide the most meaningful insights into biology. This important realization is now apparent to researchers in diverse fields, including ecology, molecular biology, and physiology. A phylogenetic framework reveals the patterns of evolution of many morphological and chemical characters, including complex pathways such as nitrogen-fixing symbioses, mustard oil production, and chemical defense mechanisms. A phylogenetic perspective also provides the basis for comparative genomics (Soltis & Soltis, 2003). Thus phylogenetic analysis of lactase concentration and PKS concentration in the *Lactobacillus* sp. were done. The species

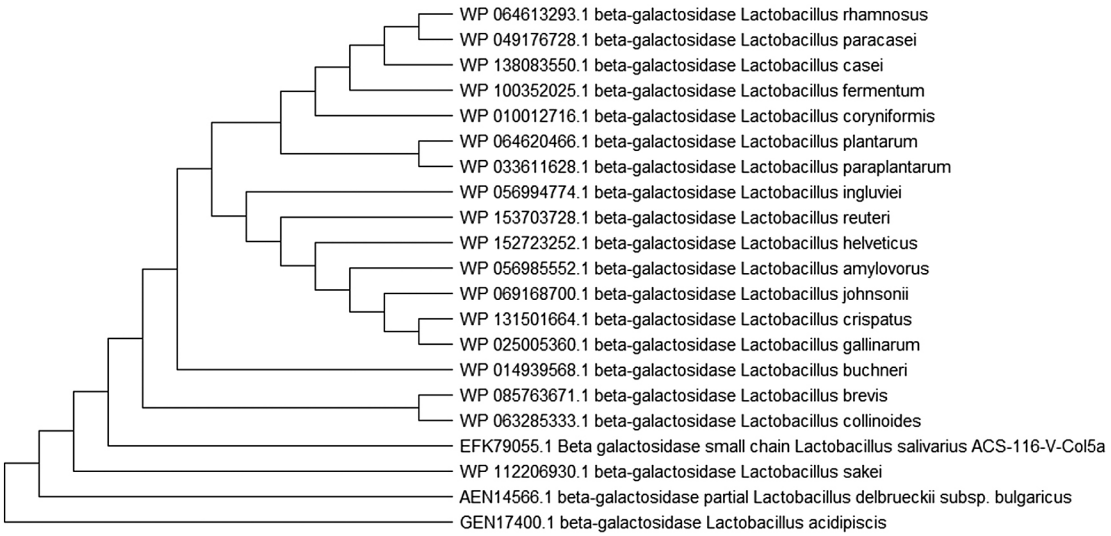


FIGURE 7.1 Phylogenetic tree of lactase concentration in *Lactobacillus* sp. by using MEGA X.

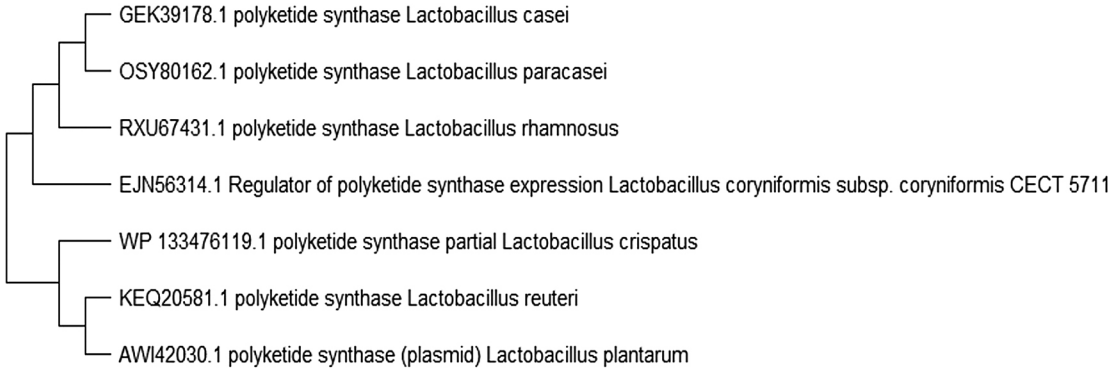


FIGURE 7.2 Phylogenetic tree of polyketide synthase (PKS) concentration in *Lactobacillus* sp. by MEGA X.

L. rhamnosus, *L. paracasei*, *L. casei*, *L. fermentum*, and *L. coryniformis* were found to have more lactase concentration compared to the rest of the *Lactobacillus* sp. (Fig. 7.1). Species, including *L. casei*, *L. paracasei*, *L. rhamnosus*, *L. coryniformis*, and *L. crispatus*, were found to have more PKS concentration in comparison with other species of *Lactobacillus* (Fig. 7.2).

11 pH concentration

pH concentration of all the six types of *Lactobacillus* species that were found to be in the top of the phylogenetic tree of both lactase and PKS concentration were as follows: *L. rhamnosus*—pH 2 (Corcoran, Stanton, Fitzgerald, & Ross, 2005); *L. paracasei*—pH 2 (Xu et al., 2019); *L. coryniformis*—pH 2–9 (Rahman et al., 2018); *L. crispatus*—pH 3.5–4.5 (Breshears, Edwards, Ravel, & Peterson, 2015); *L. fermentum*—pH 5.5 (Riaz, Nawaz, & Hasnain, 2010); and *L. casei*—pH6.4 (Lebaka, Wee, Narala, & Joshi, 2018).

12 Conclusion

Probiotic organisms are crucial for the maintenance of balance of human intestinal microbiota. Numerous scientific reports confirm their positive effect in the host's health. Probiotic microorganisms are attributed a high therapeutic potential in, for example, obesity, insulin-resistance syndrome, type 2 diabetes, and nonalcohol hepatic steatosis. It seems also that probiotics may be helpful in the treatment of irritable bowel syndrome, enteritis, bacterial infections, and various gastrointestinal

disorders and diarrhea (Markowiak & Śliżewska, 2017). Among the vast natural products (NPs) produced by *Lactobacillus* species, polyketides, nonribosomal peptides, terpenes, and alkaloids exist in diverse chemical forms and are of great interest, especially in pharmacology.

These groups of SMs are synthesized by PKSs, NRPSs, terpene synthases/cyclases, and dimethylallyl tryptophan synthases, respectively (Soltani, 2016).

Polyketides and nonribosomal peptides represent two large families of NPs with diverse structures and important functions. They are synthesized by PKS and NRPS, respectively. Since they also show antimicrobial properties (Zhang et al., 2014), they tend to be more congenial for the human use.

Thus according to the phylogenetic tree analysis and the pH study, keeping the pH of the stomach as an underpin priority, the best compatible probiotic was found to be in the following order: (1) *L. rhamnosus* with a pH of 2, (2) *L. paracasei* with a pH of 2, (3) *L. coryniformis* with pH ranging from 2 to 9, (4) *L. crispatus* with pH ranging from 3.5 to 4.5, and (5) *L. fermentum* with a pH of 5.5.

Acknowledgment

We are indebted to Prof N. Thajuddin, Programme Coordinator, School of Life Sciences, Bharathidasan University, Tiruchirappalli for encouraging me to write this chapter. We also acknowledge the financial assistance of the Department of Science and Technology, Government of India, under DST-Promotion of University Research and Scientific Excellence (PURSE) scheme-Phase II, Rashtriya Uchchatar Shiksha Abhiyan (RUSA)-2.O.

References

Anand, S., Prasad, M. V. R., Yadav, G., Kumar, N., Shehara, J., Ansari, M. Z., et al. (2010). SBSPKS: Structure based sequence analysis of polyketide synthases. *Nucleic Acids Research*, *38*(2), W487–W496.

Benson, D. A., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., & Wheeler, D. L. (2008). GenBank. *Nucleic Acids Research*, *36*, D25.

Blin, K., Wolf, T., Chevrette, M. G., Lu, X., Schwalen, C. J., Kautsar, S. A., et al. (2017). antiSMASH 4.0—Improvements in chemistry prediction and gene cluster boundary identification. *Nucleic Acids Research*, *45*(W1), W36–W41.

Breshears, L. M., Edwards, V. L., Ravel, J., & Peterson, M. L. (2015). *Lactobacillus crispatus* inhibits growth of *Gardnerella vaginalis* and *Neisseria gonorrhoeae* on a porcine vaginal mucosa model. *BMC Microbiology*, *15*, 276. doi: 10.1186/s12866-015-0608-0.

Cai, W., & Zhang, W. (2018). Engineering modular polyketide synthases for production of biofuels and industrial chemicals. *Current Opinion in Biotechnology*, *50*, 32–38. doi: 10.1016/j.copbio.2017.08.017.

Corcoran, B. M., Stanton, C., Fitzgerald, G. F., & Ross, R. P. (2005). Survival of probiotic lactobacilli in acidic environments is enhanced in the presence of metabolizable sugars. *Applied and Environmental Microbiology*, *71*(6), 3060–3067. doi: 10.1128/AEM.71.6.3060-3067.2005.

Fijan, S., & Šostar-Turk, S. (2012). Hospital textiles, are they a possible vehicle for healthcare-associated infections? *International Journal of Environmental Research and Public Health*, *9*, 3330–3343. 1660-4601; doi:10.3390/ijerph9093330.

Fijan, S. (2014). Microorganisms with claimed probiotic properties: An overview of recent literature. *International Journal of Environmental Research and Public Health*, *11*, 4745–4767. doi: 10.3390/ijerph110504745.

Gruber, I., Heudorf, U., Werner, G., Pfeifer, Y., Imirzalioglu, C., Ackermann, H., et al. (2013). Multidrug-resistant bacteria in geriatric clinics, nursing homes, and ambulant care—Prevalence and risk factors. *International Journal of Medical Microbiology*, *303*, 405–409. doi: 10.1016/j.ijmm.2013.05.002.

Holzapfel, W. H., Haberer, P., Geisen, R., Björkroth, J., & Schillinger, U. (2001). Taxonomy and important features of probiotic microorganisms in food and nutrition. *American Journal of Clinical Nutrition*, *73*(2), 365S–373S.

Kechagia, M., Dimitrios, B., Stavroula, K., Dimitra, D., Konstantina, G., Nikoletta, S., et al. (2013). Health benefits of probiotics: A review. *ISRN Nutrition*, *2013*, 7. doi: 10.5402/2013/481651. Article ID 481651.

Lebaka, V. R., Wee, Y. J., Narala, V. R., & Joshi, V. K. (2018). Chapter 4: Development of new probiotic foods—A case study on probiotic juices. In *Therapeutic, probiotic, and unconventional food* (pp. 55–78). Elsevier Inc..

Lye, H. S., Balakrishnan, K., Thiagarajah, K., Mohd Ismail, N. I., & Ooi., S. Y. (2016). Beneficial properties of probiotics. *Tropical Life Sciences Research*, *27*(2), 73–90. doi: 10.21315/tlsr2016.27.2.6.

Markowiak, P., & Śliżewska, K. (2017). Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients*, *1021*doi: 10.3390/nu9091021.

Miller, B. R., & Gulick, A. M. (2016). Structural biology of nonribosomal peptide synthetases. *Nonribosomal peptide and polyketide biosynthesis: Methods and protocols. Methods in molecular biology* (Vol. 1401). New York: Springer Science+Business Mediadoi: 10.1007/978-1-4939-3375-4_1.

Özçam, M., Tocmo, R., Oh, J. H., Afrazi, A., Mezrich, J. D., Roos, S., et al. (2018). Gut symbionts *Lactobacillus reuteri* R2lc and 2010 encode a polyketide synthase cluster that activates the mammalian aryl hydrocarbon receptor. *Applied and Environmental Microbiology*, 1–48. doi: 10.1128/AEM.01661-18.

Rahman, M. M., Aktar, S., Faruk, M. O., Uddin, M. S., Ferdouse, M. J., & Anwar, N. (2018). Probiotic potentiality of *Lactobacillus coryniformis* subsp. torquens MTi1 and *Lactobacillus coryniformis* MTi2 Isolated from intestine of *Nile tilapia*: An in vitro evaluation. *Journal of pure and Applied Microbiology*, *12*(3), . http://dx.doi.org/10.22207/JPAM.12.3.01.

Riaz, S., Nawaz, S. K., & Hasnain, S. (2010). Bacteriocins produced *by L. fermentum* and *L. acidophilus* can inhibit cephalosporin resistant *E. coli*. *Brazilian Journal of Microbiology*, *41*, 643–648. doi: 10.1590/S1517-83822010000300015.

Soltani, J. (2016). Secondary metabolite diversity of the genus *Aspergillus*: Recent advances. *New and Future Developments in Microbial Biotechnology and Bioengineering*, *Section V*, 275–292. doi: 10.1016/b978-0-444-63505-1.00035-x.

Soltis, D. E., & Soltis, P. S. (2003). The role of phylogenetics in comparative genetics. *Plant Physiology*, *132* doi: 10.1104/pp.103.022509.

Starcevic, A., Zucko, J., Simunkovic, J., Long, P. F., Cullum, J., & Hranueli, D. (2008). ClustScan: An integrated program package for the semi-automatic annotation of modular biosynthetic gene clusters and in silico prediction of novel chemical structures. *Nucleic Acids Research*, *36*(21), 6882–6892.

Xu, Y., Tian, Y., Cao, Y., Li, J., Guo, H., Su, Y., et al. (2019). Probiotic properties of *Lactobacillus paracasei* subsp. paracasei L1 and its growth performance-promotion in chicken by improving the intestinal microflora. *Frontiers in Physiology*, *10*(937), doi: 10.3389/fphys.2019.00937.

Zhang, J., Du, L., Liu, F., Xu, F., Hu, B., Venturi, V., et al. (2014). Involvement of both PKS and NRPS in antibacterial activity in *Lysobacter* enzymogenes OH11. *FEMS Microbiology Letters*, *355*, 170–176.

Chapter 8

Probiotic Polysaccharides as Toll-Like Receptor 4 Modulators—An In Silico Strategy

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1 Introduction

Innate immune system modulation is responsible for the initial action of the immune response against various pathogens (Llewellyn & Foey, 2017). Most of the autoimmune disorders arrived from the failure of an innate immune response. Rather than autoimmune disorders, some of the tumors such as colon cancer, lung cancer, melanoma, breast cancer, and prostate cancer also occurred by the failure of the innate immune system (Urban-Wojciuk et al., 2019). In lung cancer, tumor cells building up in the bone marrow and leading to weakening of immune system. Vast numbers of receptors in the cell surface, mainly Toll-like receptors (TLRs), recognize the pathogen-associated molecular patterns of different biological substances (Kuzmich et al., 2017). Particularly in cancer, TLR4 is identified as a highly important cell surface receptor than the other TLRs (Gu et al., 2018). The main role of TLR4 is to form a complex with its supplement protein myeloid differentiation factor 2 (MD2) and recognize the lipopolysaccharides (LPS) that were presented in the cell walls of Gram-negative bacteria. Binding of LPS in the active site of MD2 leads the complex formation of LPS-TLR4/MD2 that initiates downstream regulators of the innate immune system (Billod, Lacetera, Guzman-Caldentey, & Martin-Santamaria, 2016). TLR4 also had the ability to activate the well-characterized pathways responsible for tumor; for instance, the mitogen-activated protein kinase pathway and NF-κB pathway (Long et al., 2018). In addition to that expression of immunosuppressive cytokines produced by TLR4 encourages apoptosis pathway that causes resistance to cancer cells (He et al., 2007). The binding of LPS on TLR4/MD2 is an essential checkpoint for the activation of an immune response. The complex formation of TLR4/MD2 is caused by forming the polar bridge between polysaccharides that binds with TLR4/MD2 (Billod, Lacetera, Guzman-Caldentey, & Martin-Santamaria, 2016). In tumor cells, lipopolysaccharide-induced TLR4/MD2 activation supports the attack of cytotoxic T cells by stimulating important cytokines like IL-6, IL-12, B7-H1, and B7-H2 (Huang et al., 2005). Some of the research evidences indicating that probiotic-derived polysaccharides may induce the immune response via modulating TLR4 signals (Ates, 2015). In general, probiotics help the immune system to counteract host reactions in humans and animals (Fijan, 2014). Treatment and prevention of immune-related diseases using viable probiotics are frequently challenging and less feasible approaches. In particular, the exopolysaccharides produced from probiotic species have more potential benefits as an immune booster to the host organism. Probiotic polysaccharides in colorectal cancer resulted in an increased survival and tumor prevention by the modulation of TLR4 during its in vivo study (Ates, 2015). In addition to that many of the lactic acid bacteria were producing exopolysaccharides having antioxidant, antitumor, and blood cholesterol–lowering activity (Feng et al., 2019). The cell-bound exopolysaccharide from *Lactobacillus helveticus* MB2-1 is having the antitumor activity against the liver (HepG2), gastric (BGC-823), and particularly colon cancer cells (Li et al., 2015). During the treatment process, it is difficult for most probiotic species to survive and to be stable due to

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00008-3
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different environmental conditions produced by human cells (Oberoi, 2019). The main objective is to enhance the stability of the molecule and the safety of the probiotics during the therapy cycle.

In silico approaches provide more in-depth insight into the atomic level of interaction studies in the LPS-TLR4/MD2 complex. Molecular docking and molecular dynamic (MD) tactics were to predict the binding efficiency of probiotic derivatives on TLR4/MD2 (Tan & Liong, 2014). Combining probiotics with molecular approaches should make the correct decision in various therapies using probiotic supplements. This study reflects an initiative for finding the molecular interactions of probiotic polysaccharides on TLR4/MD2 signaling receptors.

2 Methodology

2.1 Dataset

The human TLR4/MD2 tetramer complex with the *Escherichia coli*-LPS receptor structure was retrieved from Protein Data Bank (PDB) (PDB ID: 3FXI) (Mishra & Pathak, 2019). The three-dimensional (3D) tetramer complex consists two monomeric units of the LPS-TLR4/MD2. Initially, we distinct a TLR4/MD2 complex in order to identify the adjuvant for the restricted binding sites located in the TLR4/MD2 interface. The ligand structures such as probiotic exopolysaccharides together with assistant compounds which help the probiotic survival were obtained from the literature (Gao, 2014; Liu, Xie, & Nie, 2020; Patel, Michaud, Singhania, Soccol, & Pandey, 2010; Soma, Williams, & Lo, 2009; Zanjani, Tarzi, Sharifan, & Mohammadi, 2014; Zeidan et al., 2017). The details of the agonists considered in our analysis were listed in Table 8.1.

TABLE 8.1 List of probiotic polysaccharides and their assistant compounds.

Probiotic polysaccharides		
S. no.	**Compounds**	**Microorganisms**
1.	Alternan	*Leuconostoc mesenteroides*
2.	Astaxanthin	*Kluyveromyces marxianus*
3.	β-D-Glucan	*Pediococcus parvulus 2.6*
4.	Capsular polysaccharides (CAPS)	*Escherichia coli K49*
5.	Cellulose	*E. coli Nissle 1917*
6.	Dextran	*L. mesenteroides*
7.	Hyaluronan	*Streptococcus equi*
8.	Inulin	*Leuconostoc citreum CW28*
9.	Kefiran	*Lactobacillus hilgardii*
10.	Levan	*Lactobacillus reuteri 121*
11.	Mutan	*Streptococcus mutans*
12.	Polygalactan	*Lactococcus lactis*
13.	Pullulan	*Aureobasidium pullulans*
14.	β-D-Galactose	*Pediococcus acidilactici*
Probiotic assistant compounds		
S. no.	**Compounds**	**PubChem_CID**
1.	Calcium alginate	44630049
2.	β-Galactan	53356679
3.	Xanthan	7107
4.	λ-Carrageenan	101231953
5.	Laminarin	439306
6.	Guar gum	44134661
7.	Chitosan	71853
8.	Kappa-carrageenan	11966249
9.	Lentinan	37723
10.	Pectin	441476

2.2 Protein and ligand preparation

3D structure of the crystallized protein was imported in the workspace of maestro by Schrödinger. Protein preparation wizard in the Schrödinger suite contains different adoptions to treat the raw protein structure. The addition of hydrogen atoms, bond order assignment, and removal of water molecules were performed during the preparation of protein. Removal of side chains and other ligands was accomplished using a 'Refine and Modify' column in the preparation wizard. Minimization option was used to resolve the clashes that occurred during the addition of hydrogens and removal of side chains in the protein structure. Finally, the protein was minimized using the OPLS_2005 force field (Shivakumar, Harder, Damm, Friesner, & Sherman, 2012) with the cutoff RMSD (root-mean-square deviation) value of 0.30 Å on hydrogens and other heavy atoms existing in the receptor molecule.

Likewise, the 3D structure of all the 24 ligand molecules was downloaded from the PubChem database and imported into a workspace of the maestro for structure preparation. The module generates single, low-energy, high-quality 3D coordinates with correct chiralities for each input structure using OPLS_2005 force field parameters (Shivakumar et al., 2012). The Epik module forecasts various tautomers and energetic penalties for the given set of ligand molecules. In order to achieve the original energy status of ligands, desalting and tautomer generations are ignored during ligand preparation. This minimized protein and ligands were then used for docking studies.

2.3 Molecular docking and prime MM/GMSA

GLIDE docking protocol was carried out to predict the binding energy of noncovalently bound ligands to TLR4/MD2. The binding pocket identification is of utmost importance prior to molecular docking. The sitemap module of the Schrödinger suite is employed for this purpose. Active sites were determined on the basis of the survival score of each site generated using a sitemap. The pocket with the highest survival score was used for grid generation. For instance, the receptor grid generation panel in the GLIDE module was utilized to construct the grid around the active site of the protein (Kanagavelu, Sunny, Balasundaram, Karuppasamy, & Veerappapillai, 2018). The van der Waals radius scaling factor of the grid box was set to 1.0 for reducing the close contact penalties between the receptor and ligands complex (Mahendran, 2017). Also, partial charges for the atoms were fixed at 0.25 cutoff range during the generation of the receptor grid.

Molecular docking was carried out using Glide extra precision (XP) mode to avoid the false-positive prediction. Moreover, XP algorithm has more stringent scoring scheme and thus facilitates the accurate prediction of ligand pose (Friesner et al., 2006). Default input parameters were set up for the XP docking, and the output file type was set to pose viewer file with the receptor molecule. We retained 10 poses per ligands for the protein during the docking studies. Besides the threshold for rejecting the minimized ligand pose set up to 50 kcal/mol. GLIDE XP rank is based on the capability of ligands to bind to the particular conformation of the protein (Ramirez & Caballero, 2016).

Further, prime MM-GBSA (molecular mechanics–generalized born surface area) analysis was performed for rescoring the ligand poses obtained in the docking analysis. Of note, this rescoring step is immense importance for the accurate prediction of binding energies (ΔG_{bind}) between the ligands and TLR4/MD2 complex. Pose viewer file was given as an input for this analysis. Variable-dielectric Generalised Born model (VSGB) mode of solvation had performed to find the best interactions among protein and ligand complex with the OPLS_2005 force field (Zhu et al., 2017). Binding free energy is calculated by the following equation:

$$\Delta G_{bind} = G_{P+L} - G_P - G_L$$

where G_P is the free energy of protein; G_L is the free energy of ligand; G_{P+L} is the free energy of the protein–ligand complex.

2.4 ADME analysis

Absorption, distribution, metabolism, and excretion analysis is forecasting the nature of chemicals that encounter the specific receptor molecule. Descriptors provided by the QikProp module of Schrödinger are helpful to explore the pharmaceutically relevant properties of the ligands against TLR4/MD2 (Jayaraj, Reteti, Kesavan, & Muthusamy, 2020). For instance, parameters associated with Lipinski's rule of 5, stars, aqueous solubility of ligands, human oral absorption rate, and other drug-like properties were determined by this program analyzed in our study.

2.5 Molecular dynamics

MD studies of docked complex structures executed using Gromacs package version 5.1.2 to gain insights into the binding pattern at the atomic level. Gromacs is an important package that utilizes Gromacs 43a1 force field to examine the vibrant motions and stability of the complex system. Initially, the topology of the ligand structures is generated by means

of PRODRG2 program (Schüttelkopf & van Aalten, 2004). The dodecahedron box is generated with a perennial border of 1.0 Å and solvated by water SPC (simple point charge) model. In order to neutralize the system, five chlorine atoms (counter ions) were supplemented into the solvation box. The steepest descent algorithm performed maximum minimization steps with an energy step size of 0.01. This process leads to disturb and eliminate the contact between the atoms through van der Waals forces. The algorithm was immobile when the maximum force was lower than 10.0 kJ/mol. Further, the minimization extended to 100 ps to balance the 0.1 bar pressure and 300K temperature of the system. Berendsen thermostat coupling was used to handle the temperature simulations inside the system. Every 2 fs interval energy of the system was updated. Hydrogen bonds and other heavy atoms were constrained by the LINCS algorithm using a holonomic model. Finally, the simulation for the system was subjected to run up to 10 ns for each complex (Low, Shamsir, Mohamed-Hussein, & Baharum, 2019). After the dynamic simulations, the trajectory output files were taken for calculating the RMSD Root-mean-square deviation (RMSD), Root-mean-square fluctuation (RMSF), and hydrogen bonds. In addition, PCA (principal component analysis) was executed to identify the latent dynamic motions of the protein with different time frames (Chen, Wang, & Zhu, 2016). In PCA the generation of covariance matrix followed through the removal of rotational and translational movement of protein atoms. The outcome of the PCA analysis produced in terms of eigenvalues that corresponds with the particular eigenvectors principal components of systems was projected as a two-dimensional representation for better understanding. Finally, FEL (free energy landscape) was used to identify the Gibbs free energy of the system. FEL generates the reports of all possible protein conformations to evaluate the conformational variability surviving in the system (Fu, Chen, Zhang, Yu, & Yang, 2017). All the representations were generated using Xmgrace package version 5.1.25.

3 Results and discussion

3.1 Molecular docking

The grid was specified using the receptor grid panel around the pocket possess the highest survival score in the sitemap analysis. For instance, the residues such as ASP29, LYS55, GLY123, ILE124, LYS125, PHE126, and ASN158 were found to present in the high scoring pockets. It is interesting to observe that importance of these residues in the TLR4/MD2 complex was also highlighted in the literature (Mishra & Pathak, 2019). Subsequently, docking analysis was carried out to explore the interaction patterns of TLR4/MD2 complex to the ligand molecules using Glide algorithm. A total of 13 probiotic exopolysaccharides alongside 10 compounds that help in the survival of probiotics (henceforth represented as Set II) were examined. The details of the polysaccharides considered in our analysis are depicted in Table 8.1. All the docking analyses are not successful. Note that only eight compounds from probiotic derivatives and seven compounds from the Set II were successfully bound with the TLR4/MD2 complex in docking analysis. The result is shown in Table 8.2. The threshold docking score of −7 kcal/mol was set in our analysis in order to explore the minimum 50% of compounds in the subsequent stage.

Docking results indicate that score varies from −4.041 to −11.496 kcal/mol. Leven resulted the highest docking score of −11.496 kcal/mol than all compounds studied in the dataset. Except for xanthan and capsular polysaccharides, all the compounds yielded binding affinity greater than −5 kcal/mol. In probiotic derivatives the compounds such as polygalactan, dextran, and kefiran were observed to have a better binding affinity with TLR4/MD2 complex. In Set II, laminarin results in a better binding affinity of −8.571 kcal/mol than other investigated compounds. Beta-D-galactan and calcium alginate also resulted in docking score better than the threshold value, −7 kcal/mol. The binding pose and binding site residues of all the compounds were shown in Fig. 8.1 and Table 8.3, respectively.

It is evident that levan binds on the hydrophobic pocket are present in the MD2 and able to maintain interaction with the residues such as ASP29, LYS55, ASN158, PHE126, LYS125, ILE124, and GLY123. Similarly, calcium alginate is also able to bind well with MD2 through residues such as ASN158, PHE126, LYS125, ILE124, GLY123, LYS122, and LYS56. In essence, the binding site residues of both levan and calcium alginate were identical to the LPS-binding pattern against TLR4/MD2 complex (Mishra & Pathak, 2019). The literature is evident that PHE126 is a crucial residue that may induce the complex formation of the LPS-TLR4/MD2 complex (Resman, Oblak, Gioannini, Weiss, & Jerala, 2014). Additionally, the binding residues of levan and calcium alginate better correlate with the binding site residues listed in the literature such as ILE46, VAL48, ILE52, ILE63, LEU74, PHE76, LEU78, GLU92, PHE104, ILE117, SER118, PHE119, SER120, PHE121, ILE124, PHE126, TYR131, and PHE151 (Mishra & Pathak, 2019). Ligand interaction diagram of top-ranked polysaccharides levan and calcium alginate was shown in Fig. 8.2.

To validate these docking scores of all compounds, prime MM-GBSA was carried out. Prime MM-GBSA produces binding free energy of the TLR4/MD2 ligand complexes. The prime score of all compounds was shown in Table 8.2. Binding free energy of the compounds was ranging from −18 to −43 kcal/mol. Most of the polysaccharide derivatives gained

TABLE 8.2 Docking score and binding energy calculations of compounds.

Compounds	Docking score (kcal/mol)	ΔG_{bind}[a]	ΔG_{bind} Coulomb[b]	ΔG_{bind} Covalent[c]	ΔG_{bind} Hbond[d]	ΔG_{bind} Solv GB[e]	ΔG_{bind} vdW[f]	Ligand strain energy
Levan	−11.496	−40.976	−42.528	2.602	−6.045	32.301	−24.703	22.804
Mutan	−5.153	−33.313	2.508	5.347	−3.657	8.997	−28.646	8.548
CAPS	−4.041	−32.529	−21.739	6.073	−2.679	18.65	−29.809	8.285
Kefiran	−7.715	−29.982	−13.786	2.931	−1.378	14.843	−21.678	3.832
β-D-Glucan	−6.055	−28.359	−34.26	4.158	−3.258	29.382	−21.242	8.12
Polygalactan	−8.803	−27.767	−90.307	4.805	−3.254	92.654	−28.704	11.785
Cellulose	−5.696	−27.077	−33.415	2.938	−3.32	27.094	−17.748	8.275
Dextran	−8.042	−23.197	−5.719	3.447	−4.007	21.314	−32.118	18.357
Calcium alginate	−7.161	−43.166	−46.675	11.989	−4.175	31.1	−31.181	1.067
β-Galactan	−7.722	−35.146	−35.155	4.701	−3.409	28.074	−25.719	11.498
Xanthan	−4.184	−34.162	−1.039	0.146	−2.432	6.673	−17.948	0.304
λ-Carrageenan	−6.702	−32.474	−26.606	4.04	−4.671	31.693	−34.448	17.513
Laminarin	−8.571	−30.11	−25.275	6.463	−4.554	24.173	−25.811	22.482
Guar gum	−6.738	−20.398	−6.577	−5.441	−6.139	29.721	−31.051	6.97
Pectin	−5.887	−18.278	−8.94	1.258	−3.545	11.933	−16.218	7.037

[a] *Binding free energy.*
[b] *Coulomb energy.*
[c] *Covalent binding energy.*
[d] *Hydrogen-bonding correction.*
[e] *Generalized Born electrostatic solvation energy.*
[f] *van der Waals energy.*

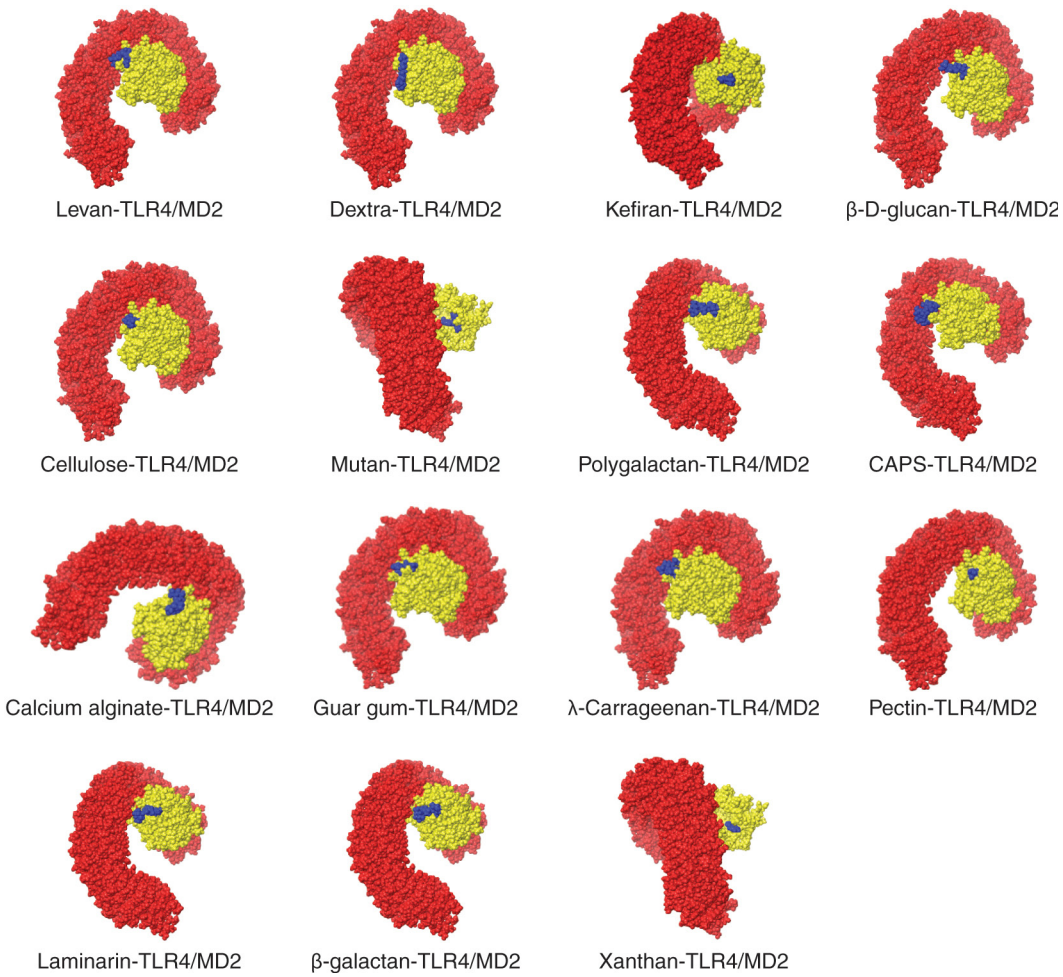


FIGURE 8.1 The binding pose of all compounds (*blue*) with TLR4 (*red*) and MD2 (*yellow*) complex.

TABLE 8.3 Interaction site residues of all compounds with TLR4/MD2.

S. no.	Compounds	Interaction residues
1.	Levan	ASP29, LYS55, GLY123, ILE124, LYS125, PHE126, ASN158
2.	Dextran	LEU54, LYS125, PHE126, SER127, LYS128
3.	Kefiran	LYS125, SER127
4.	β-D-Glucan	ASP29, GLY123, LYS125, PHE126, ASN158
5.	Cellulose	ILE124, ILE124, LYS125, ASN158
6.	Mutan	PHE121, PHE126
7.	Polygalactan	GLY123, ILE124, PHE126, ASN158
8.	CAPS	ILE124, ILE124, LYS125, ASN158
9.	Calcium alginate	LYS56, LYS122, GLY123, ILE124, LYS125, PHE126, ASN158
10.	Guar gum	ASP29, LYS55, ASN158
11.	λ-Carrageenan	LEU54, LYS55, GLY123, ILE124, LYS125, PHE126
12.	Pectin	ASP29, PHE126, ASN158
13.	Laminarin	ASP29, LYS55, GLY123, ASN158
14.	β-Galactan	GLY123, ILE124, PHE126, ASN158
15.	Xanthan	PHE126

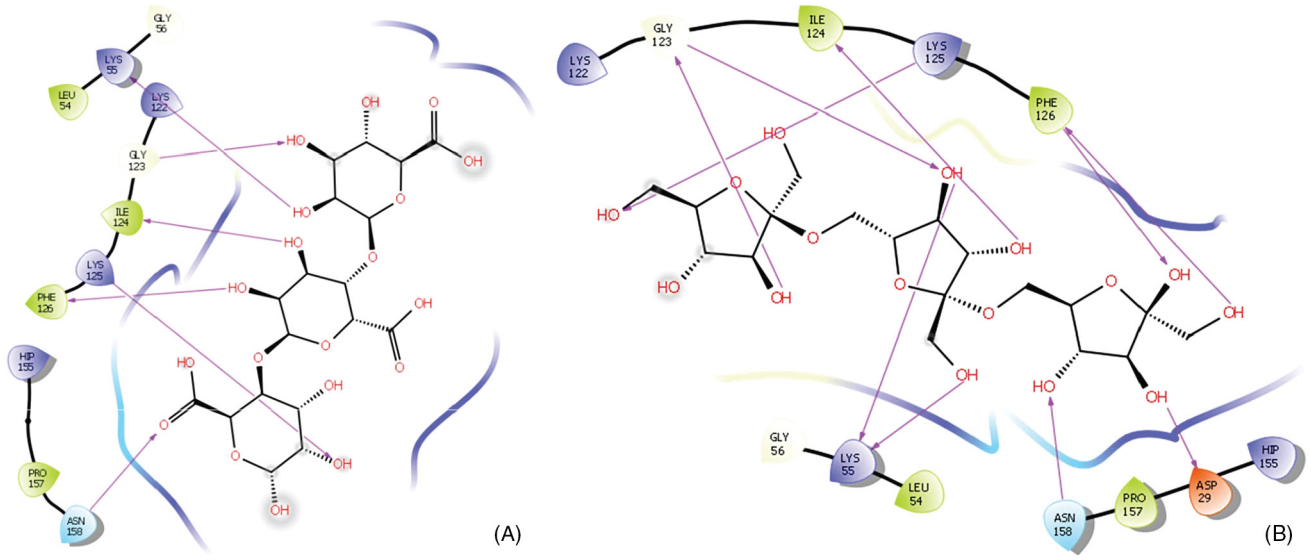


FIGURE 8.2 Ligand interactions diagram of top polysaccharides with TLR4/MD2 complex. (A) Calcium alginate and (B) levan.

lesser free energy of binding (ΔG_{bind}) than the other compounds. Also, the covalent binding energy resulted in favorable conditions for Set II compounds than the derivatives. Higher coulombic potential (−46.675) of the calcium alginate may reason for the better binding with MD2. Even levan having a higher docking score, the free energy of binding (−40.976) and coulombic potential (−42.528) were less than the calcium alginate. Next to calcium alginate, β-galactan and laminarin exhibited lesser free energy of binding in the studied compounds. Calcium alginate, β-galactan have showed better ligand strain energy than levan, laminarin, and other compounds studied in our analysis. A specific range of similarity was observed in the hydrogen bond energy terms for all the studied compounds. It indicates that the higher coulombic potential of both calcium alginate and levan stabilizes the LPS-TLR4/MD2 complex, expecting the activation of an immune response (Aaldering et al., 2017). Most of the compounds were lacking pi–pi interactions with their binding pocket residues, so the packing energy of all compounds was neglected in this analysis. Above 50% of molecules had higher (>−25) van der Waals energy that favors the binding of the polysaccharides with MD2. Based on this free energy of binding and

docking score analysis, calcium alginate, β-galactan, and levan were the best molecules that efficiently form stable interactions with MD2.

3.2 ADME analysis

The pharmacokinetic (PK) and pharmacodynamic (PD) properties of the compounds were examined by employing QikProp algorithm and the results are shown in Table 8.4. The key parameters such as activity in the central nervous system (CNS), blood–brain barrier (QPlogBB), and Lipinski parameters were explored. It is interesting to note that all investigated compounds possess an acceptable range of SASA (300–1000) and log*S* (−6.5 to 0.5). Moreover, negative CNS values highlight the inactive nature of the compounds at CNS. The majority of the compounds lay under the range of −3 to 1.2 for QPlogBB. In addition to that Lipinski's rule of 5 and Jorgensen's rule of 3 were satisfied by most of the compounds except xanthan, mutan, and pectin. Of note the predicted range of skin permeability (−8 to −10) was found to be observed for calcium alginate (Dash, 2018; Ntie-Kang, 2013). Altogether, properties listed in the tables are evident of the satisfactory PK and PD properties together with acceptable drug-likeness values of studied compounds.

3.3 Molecular dynamics

MDs simulation provides a more accurate ranking of hit compounds by considering multiple target conformations in combination with binding free energy calculations. The parameters such as RMSD, hydrogen bond analysis, PCA, and FEL were explored for the complex structures. A total of four compounds were subjected to dynamic analysis. Most importantly, we have used GSK1795091 as a reference molecule in our dynamics study to predict with high precision. The results of MD analysis for all the compounds were visualized in Figs. 8.3–8.6.

Comparatively, 10 ns of simulations were processed for each of the resulting compounds. Fig. 8.3 shows the backbone RMSD of various compounds with respect to the simulation time interval (ns). The minimal variation in the C_α-RMSD values during the simulation period suggests that the system is equilibrated and the 10 ns data are worth for further analysis. Higher variances between the RMSD values lower the protein–ligand stabilization (Baig et al., 2014). In essence, calcium alginate TLR4/MD2 complex exhibited a very less amount of deviation during the simulation and attained RMSD value of 0.3 nm than the other compounds studied in our analysis. For instance, β-galactan showed RMSD value of 0.4 nm at the end of simulation process. In fact, both levan and calcium alginate exhibited lower deviation than GSK1795091, highlighting that both compounds may bind the TLR4/MD2 complex better than the reference compound. The literature evidence also highlights the stabilization of the protein folding induced by calcium ions (Milles, Unterauer, Nicolaus, & Gaub, 2018).

Further, the hydrogen bond examination of compounds allows us to find a more potent interactive molecule with the TLR4/MD2 complex. Hydrogen bond analysis during the MD trajectory processing of compounds is shown in Fig. 8.4. All the investigated molecules have exhibited an average of four hydrogen bonds interactions with the MD2 binding site. Note that GSK1795091 was able to maintain fewer hydrogen bond interactions than the other three molecules. On the contrary, calcium alginate was able to maintain a total of eight hydrogen bond interactions during the simulation. Levan and β-galactan also exhibited a good number of hydrogen bond interactions with TLR4/MD2. The results of hydrogen bond analysis correlate well with the prime MM-GBSA and RMSD analyses.

PCA was used to examine the overall expansion of protein during dynamic variations (Mohammad et al., 2019). The covariance matrix of all the investigated compounds was shown in Fig. 8.5. It is evident from the figure that GSK-1795091-TLR4/MD2 complexes achieved much higher flexibility during the simulation than the other complexes. The higher color intensity in the covariance matrix implies greater incidence of correlated (*red*) and anticorrelated (*blue*) motions between the atoms in the complex. The trace covariance matrix of levan lesser than other compounds indicates that levan has better stability with the TLR4/MD2 even in the different time frames. β-Galactan and calcium alginate also resulted in comparatively similar trace covariance matrix values (Table 8.5), highlighting that the stability of these compounds is equivalent to that of reference compounds studied in our analysis.

Finally, global energy minima conformations of the compounds were predicted by FEL. C_α atoms of compounds were used to analyze the flexibility of the systems. Fig. 8.6 implies the FEL of resulted compounds with the TLR4/MD2 receptor. Energy derivatives concerning coordinates were calculated in FEL analysis. We also assessed global energy minima conformations, where system coordinates are generally zero. Contour map of levan demonstrates that the smaller Gibbs free energy is an indicative of higher contrast of blue color (Maisuradze, Liwo, & Scheraga, 2010). Also, levan had three global energy minima conformations. GSK1795091, calcium alginate, and β-galactan had two global energy minima conformations in their systems with only covering the least amount of portions in contours. More number of global energy minima figure out the stabilization of protein–ligand complex during the simulations. Altogether, the results obtained from

TABLE 8.4 ADME properties of docked compounds by QikProp.

Compounds	CNS[a]	Mol. MW	SASA[b]	Donor HB[c]	Accpt. HB[d]	QPlogPo/w[e]	QPlogS[f]	QPlogHERG[g]	QPlogBB[h]	QPlogKp[i]	HoA[j]	ro5[k]	ro3[l]
Levan	−2	504.441	712.383	11	21.5	−4.171	−0.629	−4.35	−4.785	−7.201	1	3	2
Dextran	−2	506.457	764.89	12	27.2	−5.789	−0.22	−4.991	−5.107	−6.633	1	3	2
Kefiran	−2	344.315	572.399	9	18.7	−3.856	−0.688	−3.928	−3.512	−6.009	1	2	2
Calcium alginate	−2	546.392	683.675	10	27.1	−5.006	−1.055	1.224	−5.288	−9.619	1	3	2
Guar gum	−2	491.184	655.726	3	18.4	−1.273	−0.594	2.456	−4.243	−7.633	1	2	1
λ-Carrageenan	−2	594.528	725.929	6	23.9	−2.958	−0.365	1.15	−4.694	−7.983	1	3	1
β-D-Glucan	−2	504.441	703.688	11	27.2	−5.644	−0.777	−4.23	−4.393	−7.224	1	3	2
Pectin	−2	194.141	344.512	4	9.5	−1.855	−0.864	−0.524	−1.786	−6.157	2	0	1
Cellulose	−2	342.299	510.09	8	18.7	−3.822	−1.282	−3.359	−2.749	−6	1	2	2
Mutan	2	309.331	561.255	1	2.25	4.644	−3.989	−5.911	0.774	−2.299	3	0	0
Polygalactan	−2	504.441	693.983	11	27.2	−5.712	−0.772	−4.095	−4.448	−7.48	1	3	2
Laminarin	−2	504.441	702.825	11	27.2	−5.613	−0.776	−4.305	−4.278	−6.922	1	3	2
β-Galactan	−2	504.441	661.225	11	27.2	−5.52	−0.795	−3.748	−3.994	−6.942	1	3	2
Xanthan	1	182.221	404.212	0	0.5	4.211	−5.529	−4.89	0.048	−0.244	3	0	0
CAPS	−2	753.704	872.782	15	38.9	−8.285	2	−5.091	−5.141	−9.2	1	3	2

[a]*Central nervous system activity.*
[b]*Solvent accessible surface area.*
[c]*Hydrogen bond donor.*
[d]*Hydrogen bond acceptor.*
[e]*Predicted water/octanol partition coefficient.*
[f]*Predicted aqueous solubility.*
[g]*Predicted IC_{50} value for blockage of HERG K+ channels.*
[h]*Predicted brain/blood partition coefficient permeability.*
[i]*Predicted skin permeability.*
[j]*Human oral absorption rate.*
[k]*Lipinski's rule of 5.*
[l]*Jorgensen's rule of 3.*

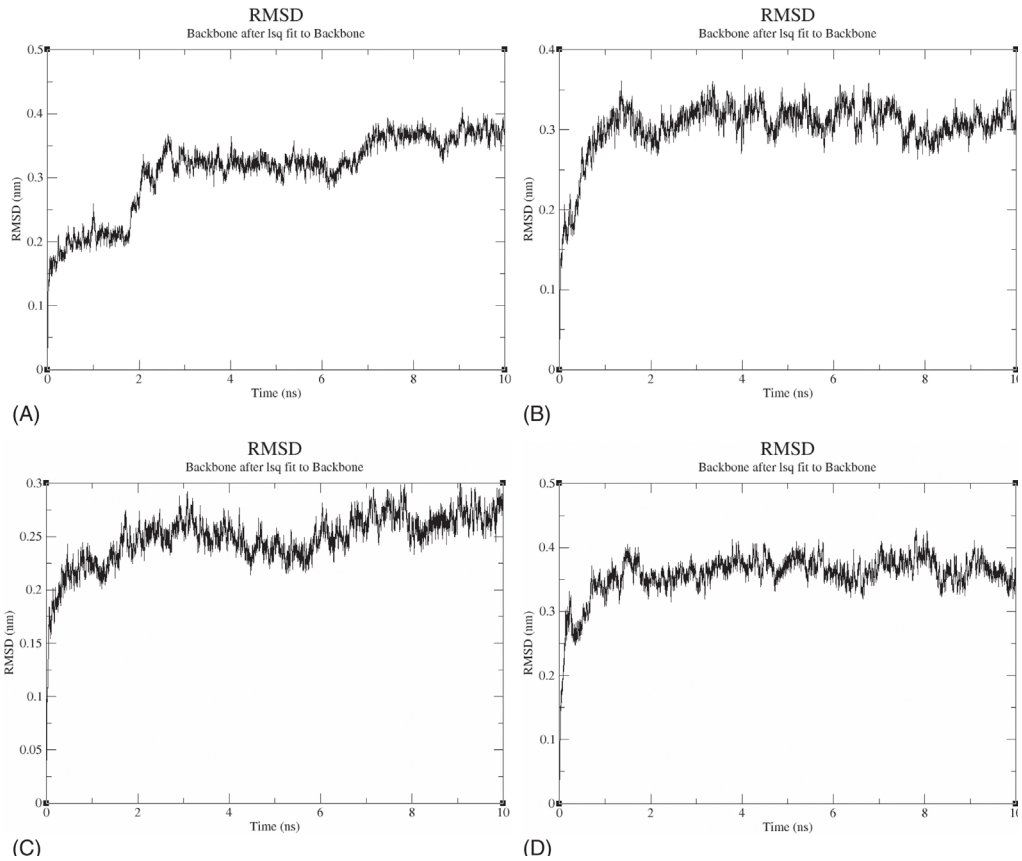


FIGURE 8.3 RMSD deviations of compounds generated by MD trajectory. (A) GSK1795091 (reference), (B) levan, (C) calcium alginate, and (D) β-galactan.

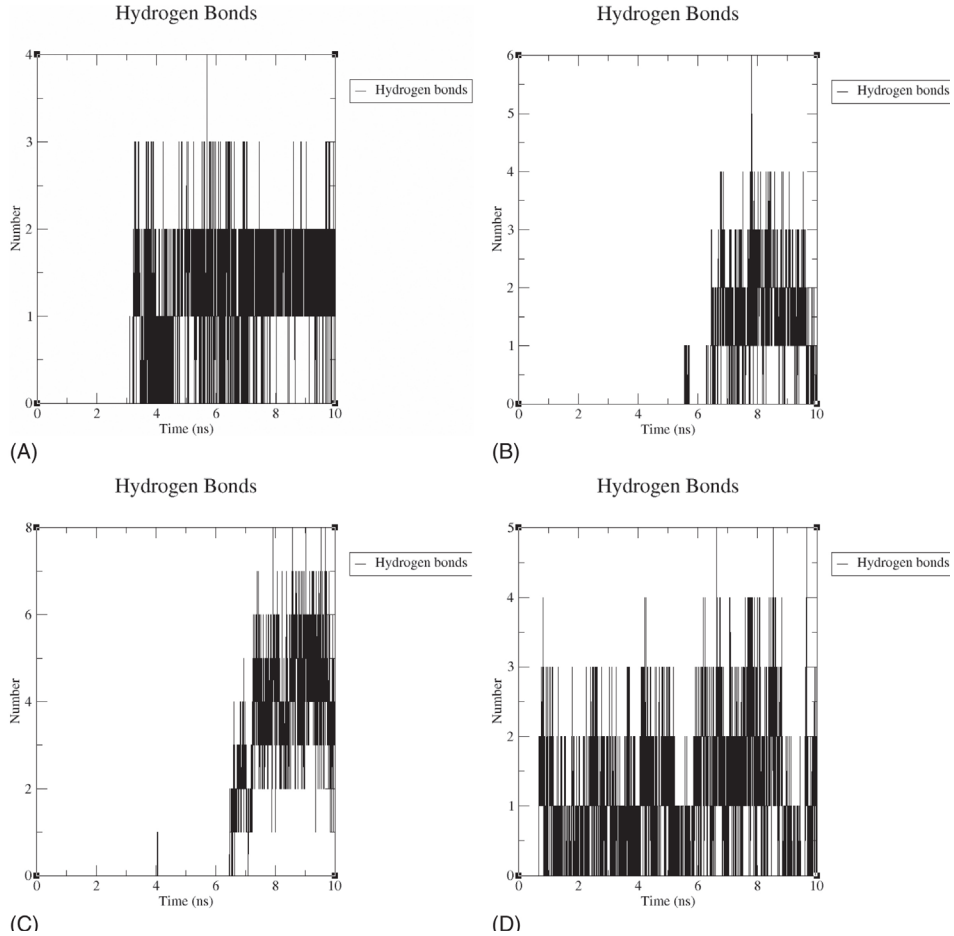


FIGURE 8.4 The intramolecular hydrogen bond interactions. (A) GSK1795091 (reference), (B) levan, (C) calcium alginate, and (D) β-galactan.

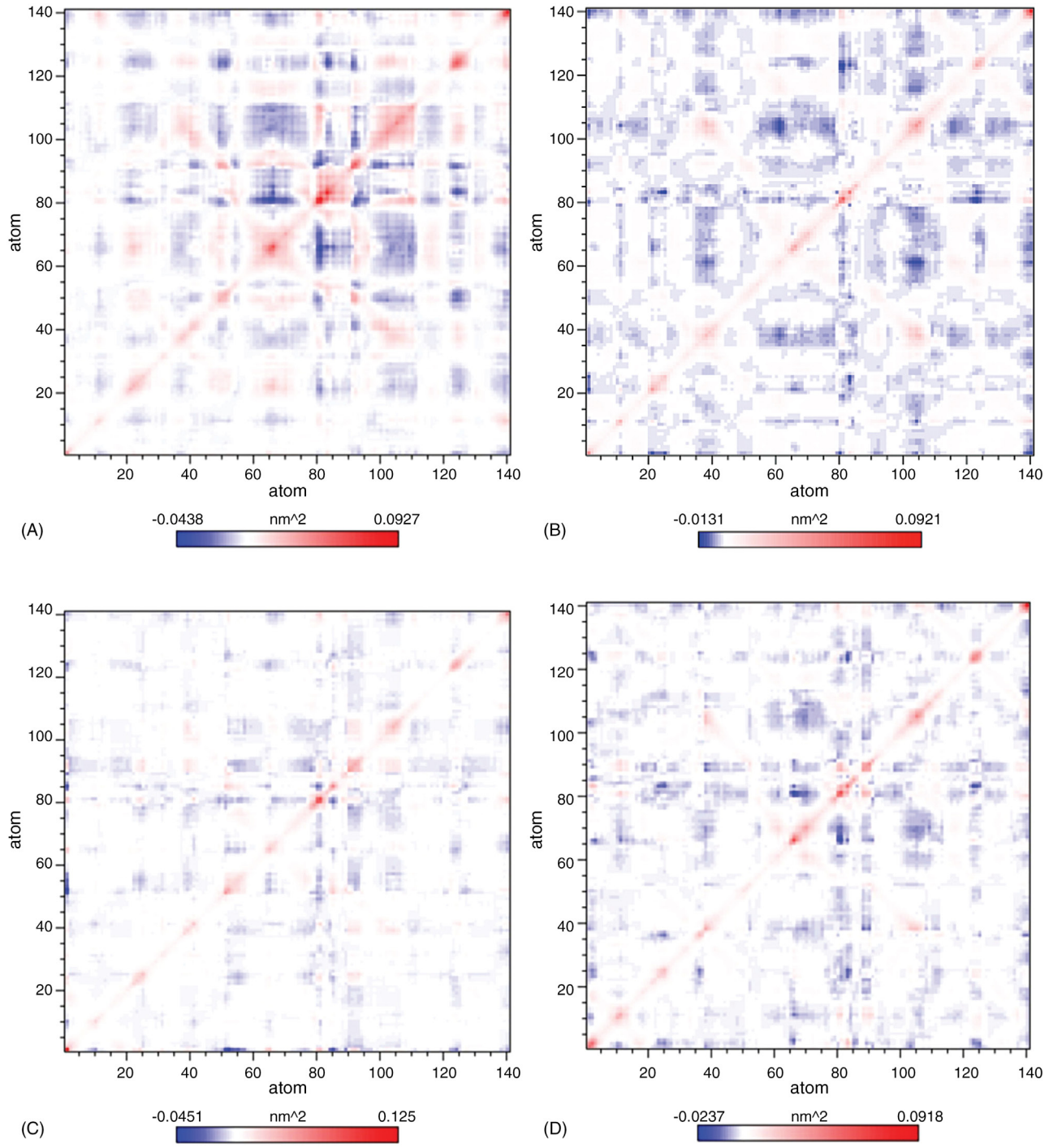


FIGURE 8.5 The covariance matrix represents the fluctuation in the atoms. (A) GSK1795091 (reference), (B) levan, (C) calcium alginate, and (D) β-galactan.

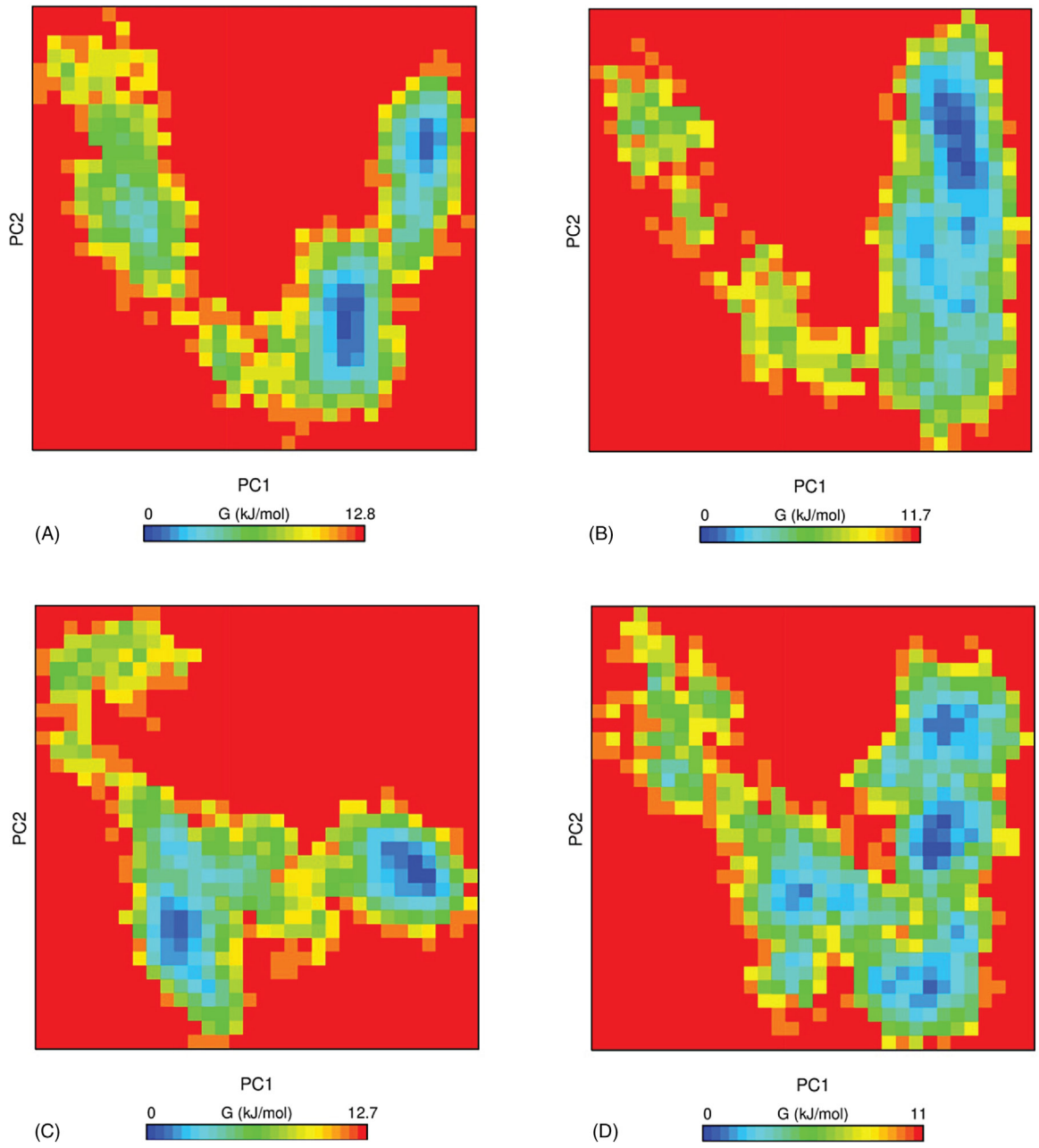


FIGURE 8.6 Gibbs free energy landscape of MD trajectories. (A) GSK1795091 (reference), (B) levan, (C) calcium alginate, and (D) β-galactan.

TABLE 8.5 Principal component analysis of compounds obtained by MD trajectory.

S. no.	Compounds	Covariance matrix (nm^2)	Trace covariance matrix (nm^2)
1.	GSK-1795091	0.0927	2.57832
2.	Levan	0.0921	1.179001
3.	Calcium alginate	0.125	2.18475
4.	β-Galactan	0.0918	2.09456

our simulation studies highlight that calcium alginate and levan exhibited better RMSD profile, hydrogen-bonding pattern, and favorable binding free energy to interact with TLR4/MD2 than reference compounds considered in the analysis.

4 Conclusion

In the present study, molecular simulation strategies were applied to accurately explore the binding efficacy of probiotic polysaccharides against the TLR4/MD2 complex. Prior to this docking analysis, it was thought that component assisting survival of the probiotic merely improves the stability that led to increased conversion. Thus a total of 10 compounds could facilitate that the probiotic survival was also examined for its binding affinity to the TLR4/MD2 protein complex. The compounds such as levan, kefiran, polygalactan, laminarin, calcium alginate, and β-galactan were showed better docking scores with the target protein in molecular docking analysis. However, kefiran, polygalactan, dextran, and laminarin did not show satisfactory binding affinity during prime MM-GBSA validation methods. On the other hand, levan, calcium alginate, and β-galactan demonstrated better binding free energy, docking score and also maintained crucial interaction with key residue (PHE126) present in the hydrophobic pocket of MD2. It is worth mentioning that the importance of PHE126 in the binding of polysaccharides on TLR4/MD2 correlates well with available literature information. Finally, MD analysis carried out to gain a deeper understanding of the binding interactions between these components. The results are evident that calcium alginate and levan had better stabilization with the TLR4/MD2 complex than other compounds considered in the analysis. Overall, we highlight that the combination of levan producing probiotic supplemented with calcium alginate may become a new potential immune adjuvant in the clinical era. It is certain that the molecular level relationship would provide useful data that are beneficial for the development of novel agonist in the near future.

Acknowledgment

The authors gratefully acknowledge the Vellore Institute of Technology, Vellore, for the support through Seed Grant for Research.

References

Aaldering, L. J., Poongavanam, V., Langkjær, N., Murugan, N. A., Jørgensen, P. T., Wengel, J., & Veedu, R. N. (2017). Development of an efficient G-quadruplex-stabilised thrombin-binding aptamer containing a three-carbon spacer molecule. *ChemBioChem*, *18*(8), 755–763.

Ates, O. (2015). Systems biology of microbial exopolysaccharides production. *Frontiers in Bioengineering and Biotechnology*, *3*, 200.

Baig, M. H., Sudhakar, D. R., Kalaiarasan, P., Subbarao, N., Wadhawa, G., Lohani, M., Khan, M. K., & Khan, A. U. (2014). Insight into the effect of inhibitor resistant S130G mutant on physico-chemical properties of SHV type beta-lactamase: A molecular dynamics study. *PLoS One*, *9*(12), e112456.

Billod, J. M., Lacetera, A., Guzman-Caldentey, J., & Martin-Santamaria, S. (2016). Computational approaches to Toll-like receptor 4 modulation. *Molecules*, *21*(8), 994.

Chen, J., Wang, J., & Zhu, W. (2016). Molecular mechanism and energy basis of conformational diversity of antibody SPE7 revealed by molecular dynamics simulation and principal component analysis. *Scientific Reports*, *6*, 36900.

Dash, R. (2018). Molecular docking and binding free energy analysis of rutin and apigetrin as galectin-1 inhibitors. *SDRP Journal of Computational Chemistry & Molecular Modelling*, *2*(2), 1–10.

Feng, Z., Yang, R., Wu, L., Tang, S., Wei, B., Guo, L., He, L., & Feng, Y. (2019). Atractylodes macrocephala polysaccharides regulate the innate immunity of colorectal cancer cells by modulating the TLR4 signaling pathway. *OncoTargets and Therapy*, *12*, 7111–7121.

Fijan, S. (2014). Microorganisms with claimed probiotic properties: An overview of recent literature. *International Journal of Environmental Research and Public Health*, *11*(5), 4745–4767.

Friesner, R., Murphy, R., Repasky, M., Frye, L., Greenwood, J., Halgren, T., et al. (2006). Extra precision glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein–ligand complexes. *Journal of Medicinal Chemistry*, *49*, 6177–6196.

Fu, M., Chen, L., Zhang, L., Yu, X., & Yang, Q. (2017). Cyclocurcumin, a curcumin derivative, exhibits immune-modulating ability and is a potential compound for the treatment of rheumatoid arthritis as predicted by the MM-PBSA method. *International Journal of Molecular Medicine*, *39*(5), 1164–1172.

Gao, C. (2014). Potential of Welan Gum as mud thickener. *Journal of Petroleum Exploration and Production Technology*, *5*(1), 109–112.

Gu, J., Liu, Y., Xie, B., Ye, P., Huang, J., & Lu, Z. (2018). Roles of Toll-like receptors: From inflammation to lung cancer progression. *Biomedical Reports*, *8*(2), 126–132.

He, W., Liu, Q., Wang, L., Chen, W., Li, N., & Cao, X. (2007). TLR4 signaling promotes immune escape of human lung cancer cells by inducing immunosuppressive cytokines and apoptosis resistance. *Molecular Immunology*, *44*(11), 2850–2859.

Huang, B., Zhao, J., Li, H., He, K., Chen, Y., Mayer, L., et al. (2005). Toll-like receptors on tumor cells facilitate evasion of immune surveillance. *Cancer Research*, *65*(12), 5009–5014.

Jayaraj, J. M., Reteti, E., Kesavan, C., & Muthusamy, K. (2020). Structural insights on vitamin D receptor and screening of new potent agonist molecules: Structure and ligand-based approach. *Journal of Biomolecular Structure and Dynamics*, *11*, 1–20.

Kanagavelu, R., Sunny, S., Balasundaram, P., Karuppasamy, R., & Veerappapillai, S. (2018). Energy based pharmacophore modelling and docking studies for determining potent inhibitor against M2 proton channel of influenza virus. *Indian Journal of Pharmaceutical Education and Research*, *52*(3), 514–524.

Kuzmich, N. N., Sivak, K. V., Chubarev, V. N., Porozov, Y. B., Savateeva-Lyubimova, T. N., & Peri, F. (2017). TLR4 signaling pathway modulators as potential therapeutics in inflammation and sepsis. *Vaccines (Basel)*, *5*(4), 34.

Li, W., Xia, X., Tang, W., Ji, J., Rui, X., Chen, X., et al. (2015). Structural characterization and anticancer activity of cell-bound exopolysaccharide from *Lactobacillus helveticus* MB2-1. *Journal of Agricultural and Food Chemistry*, *63*(13), 3454–3463.

Llewellyn, A., & Foey, A. (2017). Probiotic modulation of innate cell pathogen sensing and signaling events. *Nutrients*, *9*(10), 1156.

Long, T., Liu, Z., Shang, J., Zhou, X., Yu, S., Tian, H., et al. (2018). *Polygonatum sibiricum* polysaccharides play anti-cancer effect through TLR4-MAPK/NF-κB signaling pathways. *International Journal of Biological Macromolecules*, *111*, 813–821.

Low, C. F., Shamsir, M. S., Mohamed-Hussein, Z. A., & Baharum, S. N. (2019). Evaluation of potential molecular interaction between quorum sensing receptor, LuxP and grouper fatty acids: In-silico screening and simulation. *PeerJ*, *7*, e6568.

Mahendran, R. (2017). Molecular modeling and identification of inhibitors against human mitochondrial thymidine kinase 2. *Asian Journal of Pharmaceutical and Clinical Research*, *10*(5), 103.

Maisuradze, G. G., Liwo, A., & Scheraga, H. A. (2010). Relation between free energy landscapes of proteins and dynamics. *Journal of Chemical Theory and Computation*, *6*(2), 583–595.

Milles, L. F., Unterauer, E. M., Nicolaus, T., & Gaub, H. E. (2018). Calcium stabilizes the strongest protein fold. *Nature Communications*, *9*(1), 4764.

Mishra, V., & Pathak, C. (2019). Structural insights into pharmacophore-assisted in silico identification of protein–protein interaction inhibitors for inhibition of human Toll-like receptor 4—Myeloid differentiation factor-2 (hTLR4-MD-2) complex. *Journal of Biomolecular Structure and Dynamics*, *37*(8), 1968–1991.

Mohammad, T., Khan, F. I., Lobb, K. A., Islam, A., Ahmad, F., & Hassan, M. I. (2019). Identification and evaluation of bioactive natural products as potential inhibitors of human microtubule affinity-regulating kinase 4 (MARK4). *Journal of Biomolecular Structure and Dynamics*, *37*(7), 1813–1829.

Ntie-Kang, F. (2013). An in silico evaluation of the ADMET profile of the StreptomeDB database. *SpringerPlus*, *2*(1), 353.

Oberoi, K. (2019). Microencapsulation: An overview for the survival of probiotic bacteria. *Journal of Microbiology, Biotechnology and Food Sciences*, *9*(2), 280–287.

Patel, A. K., Michaud, P., Singhania, R. R., Soccol, C. R., & Pandey, A. (2010). Polysaccharides from probiotics: New developments as food additives. *Food Technology and Biotechnology*, *48*(4), 451–463.

Ramirez, D., & Caballero, J. (2016). Is it reliable to use common molecular docking methods for comparing the binding affinities of enantiomer pairs for their protein target? *International Journal of Molecular Sciences*, *17*(4), 525.

Resman, N., Oblak, A., Gioannini, T. L., Weiss, J. P., & Jerala, R. (2014). Tetraacylated lipid A and paclitaxel-selective activation of TLR4/MD-2 conferred through hydrophobic interactions. *Journal of Immunology*, *192*(4), 1887–1895.

Schüttelkopf, A. W., & van Aalten, D. M. (2004). PRODRG: A tool for high-throughput crystallography of protein-ligand complexes. *Acta Crystallogr D Biol Crystallogr*, *60*(Pt 8), 1355–1363.

Shivakumar, D., Harder, E., Damm, W., Friesner, R. A., & Sherman, W. (2012). Improving the prediction of absolute solvation free energies using the next generation OPLS force field. *Journal of Chemical Theory and Compututation*, *8*(8), 2553–2558.

Soma, P. K., Williams, P. D., & Lo, Y. M. (2009). Advancements in non-starch polysaccharides research for frozen foods and microencapsulation of probiotics. *Frontiers of Chemical Engineering in China*, *3*(4), 413–426.

Tan, P. L., & Liong, M. T. (2014). In silico approaches for probiotic-derived bioactives. *Trends in Biotechnology*, *32*(12), 599–601.

Urban-Wojciuk, Z., Khan, M. M., Oyler, B. L., Fahraeus, R., Marek-Trzonkowska, N., Nita-Lazar, A., et al. (2019). The role of TLRs in anti-cancer immunity and tumor rejection. *Frontiers Immunology*, *10*, 2388.

Zanjani, M. A. K., Tarzi, B. G., Sharifan, A., & Mohammadi, N. (2014). Microencapsulation of probiotics by calcium alginate-gelatinized starch with chitosan coating and evaluation of survival in simulated human gastro-intestinal condition. *Iranian Journal of Pharmaceutical Research*, *13*(3), 843.

Zeidan, A. A., Poulsen, V. K., Janzen, T., Buldo, P., Derkx, P. M. F., Oregaard, G., et al. (2017). Polysaccharide production by lactic acid bacteria: From genes to industrial applications. *FEMS Microbiology Reviews*, *41*(Supp_1), S168–S200.

Zhu, X., Zhou, L., Zhong, L., Dai, D., Hong, M., You, R., et al. (2017). Exploration of potential RSK2 inhibitors by pharmacophore modelling, structure-based 3D-QSAR, molecular docking study and molecular dynamics simulation. *Molecular Simulation*, *43*(7), 534–547.

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Part III

Quality and Nutrition of Probiotics

9. Prebiotics Mechanism of Action: An Over View 137
10. Synbiotics in Nutrition 149
11. Role of Probiotic Microbes Exerting Nutritional Properties 163

Chapter 9

Prebiotics Mechanism of Action: An Over View

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1 Introduction

Ever since the discovery by Gibson and Roberfroid in 1995, prebiotics have attracted greater attention owing to its health and clinical benefits. Prebiotics are nondigestible short-chain carbohydrates known as oligosaccharide that selectively stimulate the activity of a group of beneficial bacteria. The fermenting profile and dosage of the particular prebiotics is critical in determining its health effects. The gut microbiota is a composition of both beneficial and harmful microbes that acquired their energy from dietary macromolecules, namely, polysaccharide, protein, oligosaccharide, peptides, and glycoproteins. Presently, these dietary components undergo gut microbes-aided fermentation leading to the ultimate end production of short-chain fatty acids (SCFAs). Among a few major SCFAs, propionate, acetate, and butyrate are produced by the gut microbes (Al-Sheraji et al., 2013; Nagpal et al., 2018).

Generally, moderate levels of prebiotics are made available in human gut through consuming various fruits and vegetables such as onion, banana, garlic, and asparagus. However, the low levels of prebiotics found in these food sources are not sufficient to impose any considerable consequences on the composition of intestinal microflora (Manning & Gibson, 2007; Markowiak & Slizewska, 2017). Prebiotics are commonly a mixture of indigestible oligosaccharide except inulin, which is categorized as carbohydrates with a combination of polysaccharide and fructo-oligosaccharide (FOS) (Gibson, Ottaway, & Rastall, 2000; Manning & Gibson, 2007). The widely used prebiotics are FOS, galacto-oligosaccharide (GOS), and *trans*-galacto-oligosaccharide. These are renowned for their multifaceted effects in human body. For instance, the propionate-stimulated T helper-2 and dendritic cells are found in the airways and bone marrow, respectively (Trompette et al., 2014; Stinson, Payne, & Keelan, 2017). Additionally, the SCFAs decreased the pH of the gut and promote the growth and survival of beneficial bacteria in the colon (Hernot et al., 2009; Zhou, Zhang, Zheng, Chen, & Yang, 2013). Peptidoglycan, a prebiotic effectively involved in stimulating the specific immune response in host against variety of pathogens (Stinson et al., 2017; Clarke et al., 2010). Whatever may be the target sites, the prebiotics produced in the intestine diffuse to the blood circulation through enterocytes and reach the distant site organs (Den Besten et al., 2013) (Fig. 9.1). Considering the beneficial effects and multifaceted mode of action, prebiotics are supplemented in various dairy and bakery products, which also substitute sugar, improve taste, texture, and stabilize foam (Franck, 2002).

Although several health benefits are reported for prebiotics, their mode of action is still critical to illustrate. A plethora of studies demonstrated that the various prebiotics can facilitate in attenuating the severity of several diseases, namely, inflammatory bowel disease, diabetes, neuronal disorder, and other infections (Maguire & Maguire, 2019) in animal models (Gerards, Leodolter, Glasbrenner, & Malfertheiner, 2001). Since prebiotics are majorly involved in health and food sector, it requires a critical insight on their action mechanisms. Thus health professionals, researchers, and consumers have greater interest in investigating the other benefits of prebiotics. Healthy diets with all essential nutrients and prebiotics mitigate lifestyle diseases by proportionately maintaining the beneficial microbial populations that regulate the fermentation of nondigestible substances. In addition the balanced prebiotics in diet may fortify the immune system that reduces the threat

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00009-5
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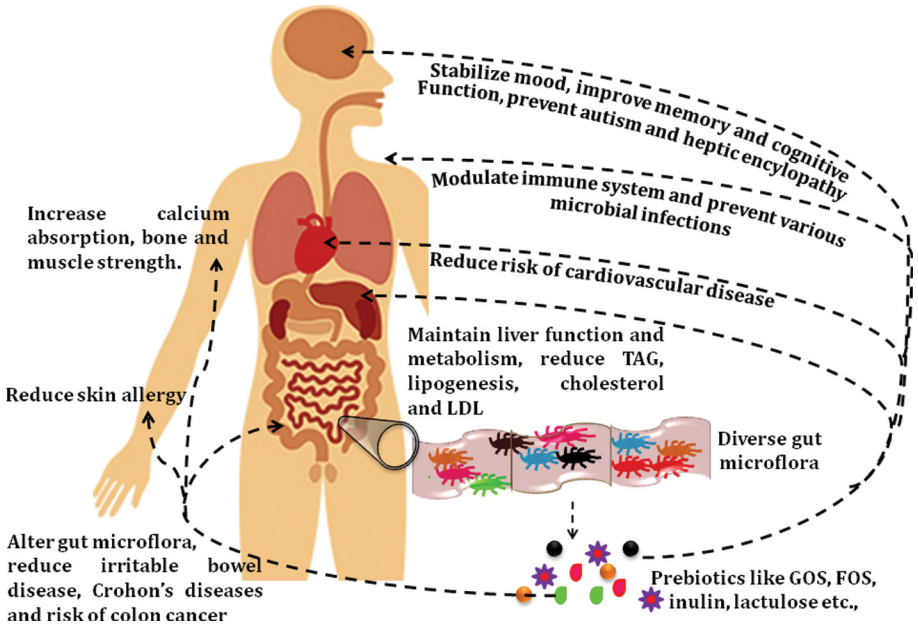


FIGURE 9.1 Mechanism of action of prebiotics in maintaining health against various diseases. Prebiotics showing protective effect on gastrointestinal system, liver, heart, and other parts of the body.

of several other inflammatory diseases and dyspepsia (Duncan & Flint, 2013). This chapter summarizes the various aspects of prebiotics mode/mechanisms of actions to promote health benefits in mankind.

2 Mechanism of prebiotics in treating constipation

Prebiotics has been clinically accepted and recommended as a dietary supplement for anticonstipation efficacy. Several carbohydrate and prebiotics are getting fermented in the large intestine and release fermentation gases. These accumulated gases increased the intestinal volume and diminished the transport time of assimilated food in intestine. The laxative environment provided by carbohydrate in large intestine and reduced travel time of digested food improve the bowel habit and render relief from constipation (Cummings, 1994). Moreover, carbohydrates improve the intestinal motility and reduce transit time by hydrating and increasing acid production in the intestine (Den Hond, Geypens, & Ghoos, 2000). In a clinical study by Cummings, Christie, & Cole (2001), individuals undergo FOS diet had a lesser occurrence of traveler's diarrhea (Cummings et al., 2001).

3 Mechanism of action of prebiotics in maintaining intestinal pH

The fermentation process of protein and carbohydrate releases ammonia and acid, respectively. Meanwhile, few intestinal-associated diseases like irritable bowel disease (IBS) and Crohn's disease (CD) are also documented to increase the pH of the intestine. Thus reducing the pH of the intestine is more helpful to the patients to attenuate the symptoms of these diseases. Various mixture of prebiotic carbohydrates underwent a fermentation process and stimulated the population of beneficial bacteria that helps in regulating the metabolism and pH of the intestine (Hemarajata & Versalovic, 2013; Markowiak & Slizewska, 2017).

4 Mechanism of action of prebiotics in maintaining lipid metabolism

Food industries seek to develop prebiotics functional foods in order to alter lipid contents such as triglycerides (TGs) and cholesterols in blood. However, research on lipid metabolism does not show any successful outcome, while some studies deciphered that the consumption of prebiotics decreased total cholesterol (TC), low density lipoprotein (LDL), and fasting TG levels (Jackson, Taylor, Clohessy, & Williams, 1999; Ooi & Liong, 2010; Sharma & Puri, 2015). In addition, dietary supplement of FOS has evidently shown to decrease the de novo synthesis of TGs in liver. Altogether, the decrease in TC, LDL, and TG may alleviate the risk of cardiovascular diseases (CVDs) (Delzenne & Kok, 1999; Van Loo, 2004).

5 Mechanism of action of prebiotics as anticarcinogenic agents

Most of the bacterial species dwell in the colon promote tumor through metabolism of food components. A plethora of studies documented antitumor efficacy in various in vivo models. For instance, in a rat model inulin inhibits aberrant crypt foci (ACF), a renowned biomarker of colon cancer (Reddy, Hamid, & Rao, 1997). Bolognani, Rumney, Pool-Zobel, & Rowland, (2001) showed that the gavage of *Lactobacillus acidophilus* and inulin reduced ACF. The prominent decrease in ACF expression in the colon during the *L. acidophilus* and inulin diet reduced the risk of colon cancer (Bolognani et al., 2001; Van Loo, 2004). In addition, clinical trials with *Lactobacillus rhamnosus*, *Bifidobacterium lactis*, and inulin (symbiotic therapy) have demonstrated to reduce the risk of colorectal cancer by inhibiting cell proliferation, necrosis and maintain the integrity of epithelial barrier (Pool-Zobel, 2005; Rafter et al., 2007; Candela et al., 2011). However, there are limited human studies reported in prebiotics for their anticarcinogenic effects. FOS and GOS produced SCFA-stimulated healthy bacterial population in the colon and provoked the protein as well as lipid metabolism to saccharolysis and thus reduced the carcinogenesis (Prasad, 1980; Manning & Gibson, 2004). Prebiotics may also influence the enzymes produced by gut microflora that are involved in carcinogenesis and alleviate the risks of cancer (Reddy, 1998; Al-Sheraji et al., 2013).

6 Mechanism of action of prebiotics in immunomodulation

Prebiotics have a potential to improve immune function by increasing the population of beneficial bacteria. Various studies on animal and human have demonstrated that prebiotics inhibit pathogens by enhancing the population of *Lactobacillus* and *Bifidobacteria* (Looijer-van Langen & Dieleman, 2009; Klatt et al., 2013; Stinson et al., 2017). Prebiotics can trigger the immunity by binding to the G protein-coupled receptor found in gut-associated lymphoid tissue. Thus stimulated immune response altered the inflammatory cytokines and decreased the accumulation of diseases-causing pathogens. Interaction of prebiotics with pattern recognition receptor attributes in maintaining the good health of an individual, by altering the expression of cytokines. The mechanism of immunomodulation by prebiotics is depicted in Fig. 9.2 . The microbial residue butyrate inhibits the proinflammatory cytokines, interferons (IFN-γ), and interleukin-2 (IL-2) in lymph nodes of rat mesenteric and in few cell lines (Cavaglieri et al., 2003; Nakamura et al., 2017; Khangwal & Shukla, 2019). In contrast, propionate and acetate stimulate the secretion of antiinflammatory cytokine IL-10. Prebiotics monomers of mannose adhere to the intestinal tract and diminished the infections by *Salmonella* (Oyofo et al., 1989; Revolledo, Ferreira, & Ferreira, 2009). In addition, mannose binds to the virulence factor type 1fimbriae and inhibits the infection (Oyofo et al., 1989). The degree of polymerization (DP) of prebiotics is a key factor to affect the production of cytokines in CD4+ T cells (Gao et al., 2017). The range of DP (DP4, DP8, DP16) of prebiotics increased the population of lactobacilli in the larger intestine triggering the secretion of IFN-γ and IL-10 (Yadav, Singh, Puniya, & Shukla, 2016). The rat consuming oligofructose stimulated with concanavalin showed elevated levels of IL-10 and IFN-γ (Dargahi, Johnson, Donkor, Vasiljevic, & Apostolopoulos, 2019). In addition, several other health benefits of prebiotics on immunity are also explored in fish and other

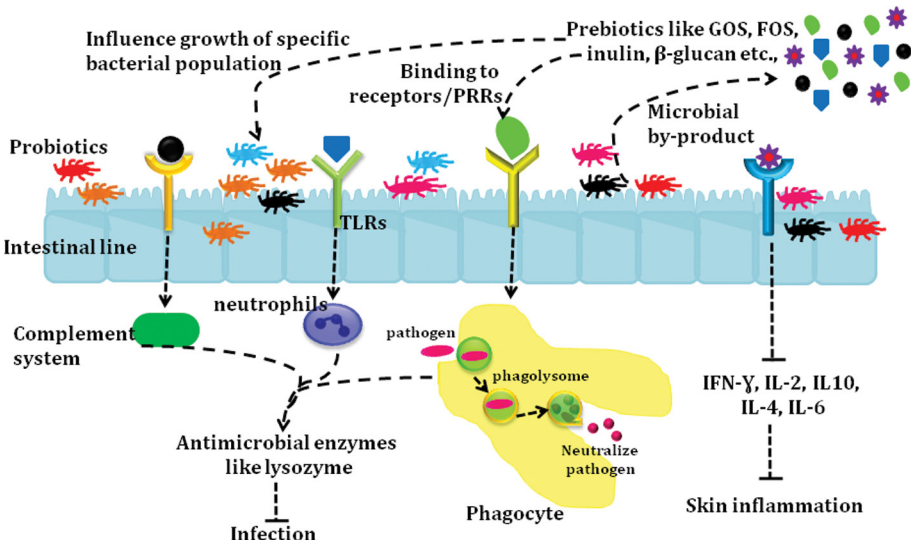


FIGURE 9.2 The illustration demonstrates the direct and indirect mechanism of action of immunomodulatory potential of prebiotics to prevent the host from various infections, allergies, and inflammations.

aquatic organisms. The immunosaccharides such as FOS, manoligosaccharide (MOS), inulin, or β-glucan boost immunity by activating phagocytes, neutrophils, complement system, and increased lysozyme (Kim, Seo, Kim, & Paik, 2011; Soleimani, Hoseinifar, Merrifield, Barati, & Abadi, 2012; Jahromi et al., 2017).

Prebiotics found in mushrooms such as proteoglycans and polysaccharides are renowned for its antitumor activity. The available glucans and heteroglycans in mushroom have a potential to activate macrophage, splenocytes, and thymocytes. A study in Wistar rat demonstrated that the inulin as a potential prebiotics, as it increased the population of *Bifidobacterium* and *Lactobacillus* spp., activates Toll-like receptors (TLRs) such as TLR-2, TLR-5, and TLR-7, which ultimately improve the immunity (Hardy, Harris, Lyon, Beal, & Foey, 2013; Mishra et al., 2019; Lepine et al., 2019). Interestingly, prebiotics in pregnant women ably pass through the placenta and affect the development of fetal immune function (Thorburn, Macia, & Mackay, 2014; Stinson et al., 2017). A study conducted in a pregnant mouse by administrating FOS influences the microflora of off-spring and consequently attenuates the skin inflammation (Fujiwara, Takemura, Watanabe, & Sonoyama, 2010). A mixture of oligofructose and inulin and FOS alone could improve antibody response against viral infections such as influenza and measles (Firmansyah, Pramita, Fassler, Haschke, & Link-Amster, 2001; Langkamp-Henken et al., 2004; Lomax et al., 2012). Apart from this, supplement of prebiotics reduced the usage of antibiotics and prevented the incidence of febrile seizure in infants (Saavedra et al., 1999; Tschernia et al., 1999). Fructans stimulate the serum IL-4, CD282+/TLR-2+ myeloid dendritic cells and TLR-2 (Clarke et al., 2016) and decrease the IL-6 and phagocytosis in monocytes and granulocytes in healthy volunteers (Guigoz, Rochat, Perruisseau-Carrier, Rochat, & Schiffrin, 2002). Notably, infants supplemented with FOS have shown reduced risk of immune diseases (Moro et al., 2006; Grüber et al., 2010). GOM improves the activity of NK cells by increasing the IL-8, IL-10, and C-reactive protein (Vulevic, Drakoularakou, Yaqoob, Tzortzis, & Gibson, 2008; Vulevic et al., 2015). It has also been reported to prevent the risks of atopic dermatitis and eczema (Moro et al., 2006; Kukkonen et al., 2007; Grüber et al., 2010).

7 Mechanism of action of prebiotics in preventing necrotizing enterocolitis (NEC)

Necrotizing enterocolitis (NEC) is a severe gastrointestinal necrosis condition in premature neonates. It causes high rate of morbidity and mortality (Patel & Denning, 2013). Prebiotics such as FOS and GOS induce the growth of *Bifidobacteria* and protect neonates from NEC and pathogenic bacterial infection (Knol et al., 2005; Kapiki et al., 2007; Patel & Denning, 2013). Moreover, SCFAs supplement to neonates enhanced feeding tolerance by improving bowel emptying and motility (Indrio et al., 2009a, 2009b). A randomized study revealed that FOS and GOS enhance the population of *Bifidobacteria* but had no significant consequence in controlling the progression of NEC (Srinivasjois, Rao, & Patole, 2009). More number of clinical trials is needed to elucidate the benefits of FOS and GOS on NEC.

8 Mechanism of action of prebiotics in preventing diabetes

Diabetes is a complex disorder associated with multiple factors such as environmental, genetics, and epigenetics. Prebiotics being significant in positively influencing the gene expression associated with metabolism, it receives huge attention in preventing diabetes. Extrapolation of a study in human intestinal microbiota elucidates the molecular link between human gut microbiome and diabetes mellitus type II (DM2) (Da avila et al., 2018). There are several prebiotics that affect the gut microbial population and alter the glycemic and insulin responses. Oligofructose acts as an antidiabetic agent and reduced the risk of DM2 in streptozotocin treated as well as in high-fat mice (Aw & Fukuda, 2018). Incorporating the prebiotics in daily diet altered the composition of gut microbiota. Notably, some prebiotics known as high-fat diet persuade glucose metabolism by modulating microbiome-gut-brain axis. Targeting this axis could aid us to prevent the obesity and diabetes (Dahiya et al., 2017). The prebiotics such as urea, trimethylamine *N*-oxide, *p*-cresyl sulfate, and 3-carboxylic acid 4-methyl-5-propyl-2-furan propionic have attenuated the abnormalities raised due to glucose homeostasis and diabetes.

9 Mechanism of action of prebiotics in preventing bowel diseases

IBS and CD are the most common bowel illness that adversely affects the gut microbiome. This deleterious effect is directly associated with the nervous system, mucosal barrier, neurotransmitters, hormones, and immune system (Menees & Chey, 2018). The interlink between microbiota-gut-brain axis in the bowel disease is illustrated in Fig. 9.3. It has been reported that when suggested prebiotics are incorporated into the diet of diseased patients, it reduced the symptoms and adverse effect by modulating the gut microflora. The commonly used prebiotics that are believed to cure IBS

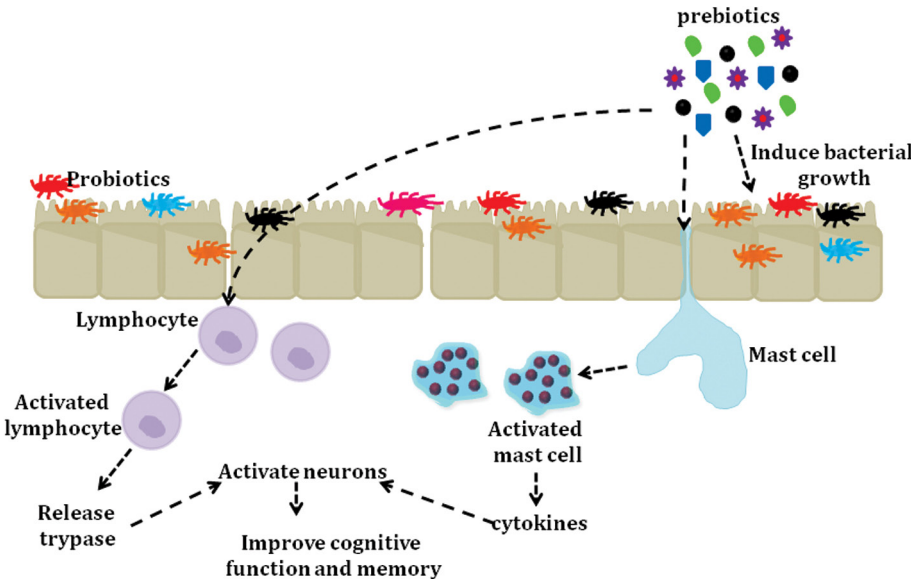


FIGURE 9.3 The graphical image explains the mechanism of action of prebiotics in improving the cognitive function and memory by maintaining the healthy gut-microbiota-brain axis.

symptoms are fructo, oligo-, di- and monosaccharides, and polyols. Prebiotic diets that eliminate gluten are also helpful in treating IBD patients. The animal as well as human case studies clearly documented the protective role of prebiotics in manipulating intestinal microflora and relief the patients from symptoms of IBS (Elison et al., 2016). Beneficial bacteria are critically involved in preventing the IBS by contributing proper prebiotics and dietary choices. A combination of 2o-fucosyllactose (2o-FL) and lacto-*N*-neotetraose (LNnT) also helped one in alleviating the IBS (Elison et al., 2016). In IBS and CD patients, it was observed that the population of *Bifidobacteria* and *Faecalibacterium prausnitzii* has been reduced in parallel with the reduction in ratio between bacteroides and firmicutes (Whelan, 2013; Wilson & Whelan, 2017). In a separate study, administration of FOS diminished the IBS symptoms at a concentration of 20, 5, and 3.5 g/day for 1, 6, and 12 weeks, respectively (Olesen & Gudmand-Hoyer, 2000; Paineau et al., 2008; Silk, Davis, Vulevic, Tzortzis, & Gibson, 2009).

10 Mechanism of prebiotics in improving nutritional absorption

Recently, intake of prebiotics is increased due to its diverse health benefits chiefly calcium and magnesium absorption. Although calcium is being absorbed in the small intestine, significant amount is also observed throughout the gut. A plethora of animal studies supplemented with inulin and oligofructose enhanced the magnesium (Tahiri et al., 2001) and calcium absorption in colon thereby strengthened bone density (Ohta et al., 2002; Coudray, Tressol, Gueux, & Rayssiguier, 2003). The clinical trial in a study with lactulose, tetraoligosaccharides, and inulin with oligosaccharide at a concentration of 5–20 g/day significantly increased the calcium absorption (Carlson, Erickson, Lloyd, & Slavin, 2018). Another notable role of prebiotics is to regulate the metabolite in the human body. The increased level of by-product interacts with the gut microflora and synergistically improves the prebiotics in the gastrointestinal tract, which, in turn, augment the membrane permeability of nutrients. The increased absorption of the nutrients by the intestinal layer may increase the level of RBCs, WBCs, and other functional proteins. A study on FOS and maltooligosaccharide in the diet increased the hemoglobin and serum protein levels (Munir, Hashim, Nor, & Marsh, 2018). The diet with combination of prebiotics (FOS and MOS) and *L. acidophilus* resists infection by *Aeromonas hydrophila* and affects the production of SCFA such as acetate, butyrate, propionate (Fernando et al., 2018). Flavonoids in combination with the dietary fibers enhance the absorption potential of the host intestine. The increased nutrients absorption modulated the metabolic activity of an individual. In a study, prolonged dietary supplement of galactooligosaccharides to mice altered the levels of 21 metabolites that include oleic acid, arachidic acid, behenic acid, etc. and affected the lipid as well as glucose metabolism of the host (Cheng et al., 2018). Difructose anhydride directly controlled the absorption of calcium and iron and thereby increased the expression of genes targeted to liver and muscle development and wound healing in intestine (Lee & Kim, 2018).

11 Mechanism of action of prebiotics in maintaining nervous system

Gastrointestinal tract is linked to central nervous system through gut-brain axis (Gaman & Kuo, 2008). Prebiotics play a crucial role in maintaining healthy gut-brain axis by altering the gut microflora. For example, administrating prebiotics with piglets reduced gray matter in brain and sequentially improved neural pruning (Mudd et al., 2016). Gut microflora affects brain through three ways such as neural, endocrine, and immune pathway (Gaman & Kuo, 2008; Grenham, Clarke, Cryan, & Dinan, 2011; Liu, Cao, & Zhang, 2015). The final product of prebiotics after process of fermentation affects the vagus nerve (Forsythe, Bienenstock, & Kunze, 2014). The FOS and GOS are reported to modulate brain-derived neurotrophic factor, neurotransmitters (D-serine), and synaptic proteins (*N*-methyl-D-aspartate, NMDA receptor, synaptophysin) (Savignac et al., 2013; Williams et al., 2016). Modulating probiotics through prebiotics can also regulate the neuroendocrine pathway. In a study, prebiotics act as a regulator of hormones and modulate the microbial population in a mice model and induce the regulation of corticosterone and adrenocorticotropic hormone (ACTH) (Sudo et al., 2004; Savignac et al., 2013). Beside neurological function, prebiotics also have the potential to control mood, memory, learning, and few psychiatric disorders by altering the composition of gut microflora and its activity (Liu et al., 2015). Increase in the levels of stress hormones leads to anxiety-like behaviors (Diaz Heijtz et al., 2011; Neufeld, Kang, Bienenstock, & Foster, 2011). A germ-free mice showed an elevated level of stress hormones, whereas administration of *Bifidobacterium infantis* normalized corticosterone and ACTH (Sudo et al., 2004). A plethora of studies on animal models and human have demonstrated the prebiotics-mediated memory improvement (Nelson, Ramberg, Best, & Sinnott, 2012). A variety of prebiotics have exhibited memory improvement potential in middle-aged adults (Best, Kemps, & Bryan, 2010; Best, Howe, Bryan, Buckley, & Scholey, 2015). Administration of arabinoxylan and arabinose enhances cognitive function and attenuates the deposition of dementia-related glial fibrillary acidic protein in mice (Han et al., 2010). A randominized and placebo-controlled study conducted by administrating FOS and GOS demonstrated the increased level of cortisol in saliva and emotional alteration in human. In addition, GOS at 5.5 g/day enhances the cortisol in saliva and improves the concentration in adult (Schmidt et al., 2015). Administration of nonstarch polysaccharide improves the recall and memory in middle-aged adult (Best et al., 2010, 2015). In another investigation, dietary supplement of inulin enriched with oligofructose helps one to enhance mood, immediate memory, recognition, and recall (Smith, 2005; Smith, Sutherland, & Hewlett, 2015). The piglets administrated with the mixture of polydextrose and GOS reduced the anxiety-like behavior, improve memory, and enhance positive social interaction (Messaoudi, Rozan, Nejdi, Hidalgo, & Desor, 2005; Mudd et al., 2016).

12 Mechanism of action of prebiotics in preventing autism

Almost 70% of the total autism population was suffered with severe gastrointestinal disorder. The spectrum of autism disorder includes chronic constipation, abdominal pain, frequent diarrhea, gastroesophageal reflux disease, bloating, disaccharide deficiencies, abnormalities in nervous system, and gut inflammation (Buie et al., 2010). In several cases the severity of the autism is correlated with gastrointestinal disorder (Adams, Johansen, Powell, Quig, & Rubin, 2011; De Angelis et al., 2013). The composition of gut microflora in autism patients was observed to be modified with depleted population of *Bifidobacterium*. Concomitantly, in children with autism showed different gut metabolite, decreased SCFAs, and increased clostridium than the healthy individuals (Adams et al., 2011; De Angelis et al., 2013). Treating autism children with wheat fiber decreased *Clostridium perfringens* and increased the population of *Bifidobacteria* in gut (Lefranc-Millot et al., 2012). In a study conducted in a rat adrenal medulla cell line, SCFA increased the expression of tyrosine hydroxylase which is critical in reducing the catecholamines (Nankova, Agarwal, MacFabe, & La Gamma, 2014). Catecholamine is a neurotransmitter that is highly expressed in the individuals with autism.

13 Mechanism of action of prebiotics in preventing hepatic encephalopathy

Hepatic encephalopathy (HE) occurs due to the improper function of liver with a condition of increased ammonia in the blood. It causes severe psychiatric and neurological impairments such as movement and speech disorders, cognitive impairment, that eventually leads to coma and death. The prebiotic lactulose decreased the blood ammonia level and cured HE (Bircher, Muller, Guggenheim, & Haemmerli, 1966; Shukla, Shukla, Mehboob, & Guha, 2011). Lactulose reduces the intestinal pH by releasing H^+ ions and converts the ammonia into ammonium; this creates a concentration gradient and aids to reuptake the ammonia from blood into gastrointestine (Elkington, Floch, & Conn, 1969). In addition, lactulose minimized the colon transit time and thus reduced the level of ammonia in the gastrointestinal tract which helps in treating the HE. Another prebiotic lactitol is as effective as lactulose in treating HE (Blanc et al., 1992; Weber, 1996).

14 Mechanism of action of prebiotics in preventing skin diseases

Previously, reports have shown that the consumption of prebiotics reduced the severity of skin allergy, namely, atopic dermatitis (Moro et al., 2006; Grüber et al., 2010). In addition, consumption of GOS for 12 weeks inhibited dehydration and erythema caused during the UV exposure in mice (Hong et al., 2015). Administration of GOS increased the dermal expression of cell adhesion and matrix formation markers such as CD44 and collagen type I. During metabolism, amino acids, phenols, and vitamins were produced in gut and transported to the skin. A type of phenol called *p*-cresol was toxic to patients suffering from kidney diseases (Kawakami, Makino, Asahara, Kato, & Onoue, 2005). A number of studies have shown that the women consuming GOS alone or along with *Bifidobacterium breve* could attenuate dehydration and keratin formation induced by phenols (Miyazaki, Masuoka, Kano, & Iizuka, 2014; Kano et al., 2013).

15 Mechanism of action of prebiotics in preventing cardiovascular diseases

CVD causes about 30% of the total death in the United States. The major reasons for this growing trend are the alteration in the eating habits and lifestyle of the people. Recently, researcher have examined whether dietary fibers and prebiotics reduced the risks of CVD. Dietary intake of prebiotics reduced the inflammatory components and lowered the risks of CVD. In addition, prebiotics indirectly prevented the CVD by reducing the cholesterol level in the human body. L-Rhamnose and lactulose altered the lipid profile with reduced level of cholesterol and apolipoprotein (Vogt, Ishii-Schrade, Pencharz, Jones, & Wolever, 2006). The administration of commercial prebiotics Bimuno GOSs for 12 weeks has decreased the circulating cholesterol, triglycerides, and total high density lipoprotein (Vulevic, Juric, Tzortzis, & Gibson, 2013). In a spate metaanalysis study, intake of glucan and FOS independently reduced the levels of triacylglyceride, TC, and LDL (Tiwari & Cummins, 2011; Brighenti, 2007). Paradoxically, prebiotics determine the lipid profile of the individual through SCFAs and acetate by converting acetate to acetyl-CoA, a substrate for fatty acid synthesis in hepatocytes (Beynen, Buechler, Van der Molen, & Geelen, 1982). However, some FOS, L-rhamnose, and SCFAs (namely, propionate and butyrate) exhibit a lipogenic effect and improve the lipid profile that ultimately reduced the risks of CVD (Wolever, Spadafora, Cunnane, & Pencharz, 1995; Mortensen, Holtug, & Rasmussen, 1988; Hernot et al., 2009).

16 Conclusion

Prebiotics have a multifaceted remedy to maintain human health; thus it attracted huge attention to protect lives against various severe diseases. Even though there are several reports demonstrated the positive effects of prebiotics on human health, the accurate long-term therapeutic designs, clinical trials, genomic investigations to confirm the health claims are still lacking. Knowledge on these aspects is critical for scientist to formulate enhanced food supplement to derive appealing procedure to control and heal disorders. Gut microbiota is a chief organ that can be fed properly on prebiotics and can exhibit multifaceted beneficial effects. Giving prominence to the minor side effects, prebiotics are recommended to all age groups. Thus designing particular, personalized prebiotics with regard to the reside gut microflora may eventually pave the way to treat certain diseases in a standardized approach. However, the concept of prebiotics on clinical trials should defeat the controversies to recommend FAO in future guidelines.

Acknowledgment

The authors thankfully acknowledge the computational and bioinformatics facility provided by the Bioinformatics Infrastructure Facility (funded by DBT, GOI; File No. BT/BI/25/012/2012, BIF). Also, the authors sincerely acknowledge UGC-SAP [Grant No. F.5-1/2018/DRS-II (SAP-II)], DST-FIST [Grant No. SR/FST/LSI-639/2015 (C)], and DST PURSE [Grant No. SR/PURSE Phase 2/38 (G)] for rendering instrumentation and infrastructure facilities. SG gratefully acknowledges UGC for Start-Up Grant (Grant No. F.30-381/2017(BSR)/F.D Diary No. 2892), Alagappa University for AURF (Ref: ALU:AURF Start-up Grant: 2018), and RUSA 2.0 [F.24-51/2014-U, Policy (TN Multi-Gen), Department of Education, Government of India].

References

Adams, J. B., Johansen, L. J., Powell, L. D., Quig, D., & Rubin, R. A. (2011). Gastrointestinal flora and gastrointestinal status in children with autism-Comparisons to typical children and correlation with autism severity. *BMC Gastroenterology*, *11*, 22.

Al-Sheraji, S. H., Ismail, A., Manap, M. Y., Mustafa, S., Yusof, R. M., & Hassan, F. A. (2013). Prebiotics as functional foods: A review. *Journal of Functional Foods*, *5*(4), 1542–1553.

Aw, W., & Fukuda, S. (2018). Understanding the role of the gut ecosystem in diabetes mellitus. *Journal of Diabetes Investigation*, *9*(1), 5–12.

Best, T., Kemps, E., & Bryan, J. (2010). Saccharide effects on cognition and well-being in middle-aged adults: A randomized controlled trial. *Developmental Neuropsychology*, *35*(1), 66–80.

Best, T., Howe, P., Bryan, J., Buckley, J., & Scholey, A. (2015). Acute effects of a dietary non-starch polysaccharide supplement on cognitive performance in healthy middle-aged adults. *Nutritional Neuroscience*, *18*(2), 76–86.

Beynen, A. C., Buechler, K. F., Van der Molen, A. J., & Geelen, M. J. (1982). The effects of lactate and acetate on fatty acid and cholesterol biosynthesis by isolated rat hepatocytes. *International Journal of Biochemistry*, *14*(3), 165–169.

Bircher, J., Muller, J., Guggenheim, P., & Haemmerli, U. P. (1966). Treatment of chronic portal-systemic encephalopathy with lactulose. *Lancet*, *1*(7443), 890–892.

Blanc, P., Daures, J. P., Rouillon, J. M., Peray, P., Pierrugues, R., Larrey, D., et al. (1992). Lactitol or lactulose in the treatment of chronic hepatic encephalopathy: Results of a meta-analysis. *Hepatology*, *15*(2), 222–228.

Bolognani, F., Rumney, C. J., Pool-Zobel, B. L., & Rowland, I. R. (2001). Effect of lactobacilli, bifidobacteria and inulin on the formation of aberrant crypt foci in rats. *European Journal of Nutrition*, *40*(6), 293–300.

Brighenti, F. (2007). Dietary fructans and serum triacylglycerols: A meta-analysis of randomized controlled trials. *Journal of Nutrition*, *137*(11), 2552S–2556S.

Buie, T., Campbell, D. B., Fuchs, G. J., Furuta, G. T., 3rd, Levy, J., Vandewater, J., et al. (2010). Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with ASDs: A consensus report. *Pediatrics*, *125*(Suppl. 1), S1–S18.

Candela, M., Guidotti, M., Fabbri, A., Brigidi, P., Franceschi, C., & Fiorentini, C. (2011). Human intestinal microbiota: Cross-talk with the host and its potential role in colorectal cancer. *Critical Reviews in Microbiology*, *37*(1), 1–14.

Carlson, J. L., Erickson, J. M., Lloyd, B. B., & Slavin, J. L. (2018). Health effects and sources of prebiotic dietary fiber. *Current Developments in Nutrition*, *2*(3), nzy005.

Cavaglieri, C. R., Nishiyama, A., Fernandes, L. C., Curi, R., Miles, E. A., & Calder, P. C. (2003). Differential effects of short-chain fatty acids on proliferation and production of pro- and anti-inflammatory cytokines by cultured lymphocytes. *Life Sciences*, *73*(13), 1683–1690.

Cheng, W., Lu, J., Lin, W., Wei, X., Li, H., Zhao, X., et al. (2018). Effects of a galacto-oligosaccharide-rich diet on fecal microbiota and metabolite profiles in mice. *Food & Function*, *9*(3), 1612–1620.

Clarke, S. T., Green-Johnson, J. M., Brooks, S. P., Ramdath, D. D., Bercik, P., Avila, C., et al. (2016). Beta2-1 fructan supplementation alters host immune responses in a manner consistent with increased exposure to microbial components: Results from a double-blinded, randomised, cross-over study in healthy adults. *British Journal of Nutrition*, *115*(10), 1748–1759.

Clarke, T. B., Davis, K. M., Lysenko, E. S., Zhou, A. Y., Yu, Y., & Weiser, J. N. (2010). Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. *Nature Medicine*, *16*(2), 228–231.

Coudray, C., Tressol, J. C., Gueux, E., & Rayssiguier, Y. (2003). Effects of inulin-type fructans of different chain length and type of branching on intestinal absorption and balance of calcium and magnesium in rats. *European Journal of Nutrition*, *42*(2), 91–98.

Cummings, J. H. (1994). Non-starch polysaccharides (dietary fibre) including bulk laxatives in constipation. In *Constipation* (pp. 307–314). Petersfield, UK: Wrightson Biomedical.

Cummings, J. H., Christie, S., & Cole, T. J. (2001). A study of FOS in the prevention of traveler's diarrhea. *Alimentary Pharmacology & Therapeutics*, *15*(8), 1139–1145.

Da avila, L. A., Pirela, V. B., Villasmil, N. R., Cisternas, S., Díaz, W., Escobar, M. C., et al. (2018). New insights into alleviating diabetes mellitus: Role of gut microbiota and a nutrigenomic approach. In V. Waisundara (Ed.), *Diabetes Food Plan* (pp. 183–193). Rijeka: IntechOpen.

Dahiya, D. K., Renuka, M., Puniya, U. K., Shandilya, T., Dhewa, N., Kumar, S., et al. (2017). Gut microbiota modulation and its relationship with obesity using prebiotic fibers and probiotics: A review. *Frontiers in Microbiology*, *8*, 563.

Dargahi, N., Johnson, J., Donkor, O., Vasiljevic, T., & Apostolopoulos, V. (2019). Immunomodulatory effects of probiotics: Can they be used to treat allergies and autoimmune diseases? *Maturitas*, *119*, 25–38.

De Angelis, M., Piccolo, M., Vannini, L., Siragusa, S., De Giacomo, A., Serrazzanetti, D. I., et al. (2013). Fecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. *PLoS One*, *8*(10), e76993.

Delzenne, N. M., & Kok, N. N. (1999). Biochemical basis of oligofructose-induced hypolipidemia in animal models. *Journal of Nutrition*, *129*(7), 1467S–1470S.

Den Besten, G., van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D. J., & Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research*, *54*(9), 2325–2340.

Den Hond, E., Geypens, B., & Ghoos, Y. (2000). Effect of high performance chicory inulin on constipation. *Nutrition Research*, *20*(5), 731–736.

Diaz Heijtz, R., Wang, S., Anuar, F., Qian, Y., Bjorkholm, B., Samuelsson, A., et al. (2011). Normal gut microbiota modulates brain development and behavior. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(7), 3047–3052.

Duncan, S. H., & Flint, H. J. (2013). Probiotics and prebiotics and health in ageing populations. *Maturitas*, *75*(1), 44–50.

Elison, E., Vigsnaes, L. K., Rindom Krogsgaard, L., Rasmussen, J., Sorensen, N., McConnell, B., et al. (2016). Oral supplementation of healthy adults with 2´-O-fucosyllactose and lacto-N-neotetraose is well tolerated and shifts the intestinal microbiota. *British Journal of Nutrition*, *116*(8), 1356–1368.

Elkington, S. G., Floch, M. H., & Conn, H. O. (1969). Lactulose in the treatment of chronic portal-systemic encephalopathy. A double-blind clinical trial. *The New England Journal of Medicine*, *281*(8), 408–412.

Fernando, W. M. A. D. B., Flint, S. H., Ranaweera, K. K. D. S., Bamunuarachchi, A., Johnson, S. K., & Brennan, C. S. (2018). The potential synergistic behaviour of inter- and intra-genus probiotic combinations in the pattern and rate of short chain fatty acids formation during fibre fermentation. *International Journal of Food Sciences and Nutrition*, *69*(2), 144–154.

Firmansyah, A., Pramita, G., Fassler, A. C., Haschke, F., & Link-Amster, H. (2001). Improved humoral immune response to measles vaccine in infants receiving infant cereal with fructooligosaccharides. *Journal of Pediatric Gastroenterology and Nutrition*, *31*, A521.

Forsythe, P., Bienenstock, J., & Kunze, W. A. (2014). Vagal pathways for microbiome-brain-gut axis communication. *Advances in Experimental Medicine and Biology*, *817*, 115–133.

Franck, A. (2002). Technological functionality of inulin and oligofructose. *British Journal of Nutrition*, *87*(Suppl. 2), S287–S291.

Fujiwara, R., Takemura, N., Watanabe, J., & Sonoyama, K. (2010). Maternal consumption of fructo-oligosaccharide diminishes the severity of skin inflammation in offspring of Nc/Nga mice. *British Journal of Nutrition*, *103*(4), 530–538.

Gaman, A., & Kuo, B. (2008). Neuromodulatory processes of the brain-gut axis. *Neuromodulation*, *11*(4), 249–259.

Gao, K., Wang, C., Liu, L., Dou, X., Liu, J., Yuan, L., et al. (2017). Immunomodulation and signaling mechanism of *Lactobacillus rhamnosus* GG and its components on porcine intestinal epithelial cells stimulated by lipopolysaccharide. *Journal of Microbiology, Immunology and Infection*, *50*(5), 700–713.

Gerards, C., Leodolter, A., Glasbrenner, B., & Malfertheiner, P. (2001). *H. Pylori* infection and visceral hypersensitivity in patients with irritable bowel syndrome. *Digestive Diseases*, *19*(2), 170–173.

Gibson, G. R., Ottaway, P. B., & Rastall, R. (2000). *Prebiotics: New developments in functional foods*. Chandos: Food industrya and trade. https://books.google.co.in/books/about/Prebiotics.html?id=TVxGAAAACAAJ.

Grenham, S., Clarke, G., Cryan, J. F., & Dinan, T. G. (2011). Brain-gut-microbe communication in health and disease. *Frontiers in Physiology*, *2*, 94.

Grüber, C., van Stuijvenberg, M., Mosca, F., Moro, G., Chirico, G., Braegger, C. P., et al. (2010). Reduced occurrence of early atopic dermatitis because of immunoactive prebiotics among low-atopy-risk infants. *The Journal of Allergy and Clinical Immunology*, *126*(4), 791–797.

Guigoz, Y., Rochat, F., Perruisseau-Carrier, G., Rochat, I., & Schiffrin, E. (2002). Effects of oligosaccharide on the faecal flora and non-specific immune system in elderly people. *Nutrition Research*, *22*(1), 13–25.

Han, H. S., Jang, J. H., Choi, J. S., Kim, Y. J., Lee, C., Lim, S. H., et al. (2010). Water extract of *Triticum aestivum* L. and its components demonstrate protective effect in a model of vascular dementia. *Journal of Medicinal Food*, *13*(3), 572–578.

Hardy, H., Harris, J., Lyon, E., Beal, J., & Foey, A. D. (2013). Probiotics, prebiotics and immunomodulation of gut mucosal defences: Homeostasis and immunopathology. *Nutrients*, *5*(6), 1869–1912.

Hemarajata, P., & Versalovic, J. (2013). Effects of probiotics on gut microbiota: Mechanisms of intestinal immunomodulation and neuromodulation. *Therapeutic Advances in Gastroenterology*, *6*(1), 39–51.

Hernot, D. C., Boileau, T. W., Bauer, L. L., Middelbos, I. S., Murphy, M. R., Swanson, K. S., et al. (2009). *In vitro* fermentation profiles, gas production rates, and microbiota modulation as affected by certain fructans, galactooligosaccharides, and polydextrose. *Journal of Agricultural and Food Chemistry*, *57*(4), 1354–1361.

Hong, K. B., Jeong, M., Han, K. S., Hwan Kim, J., Park, Y., & Suh, H. J. (2015). Photoprotective effects of galacto-oligosaccharide and/or *Bifidobacterium longum* supplementation against skin damage induced by ultraviolet irradiation in hairless mice. *International Journal of Food Sciences and Nutrition*, *66*(8), 923–930.

Indrio, F., Riezzo, G., Raimondi, F., Bisceglia, M., Cavallo, L., & Francavilla, R. (2009a). Effects of probiotic and prebiotic on gastrointestinal motility in newborns. *Journal of Physiology and Pharmacology*, *60*(Suppl. 6), 27–31.

Indrio, F., Riezzo, G., Raimondi, F., Francavilla, R., Montagna, O., Valenzano, M. L., et al. (2009b). Prebiotics improve gastric motility and gastric electrical activity in preterm newborns. *Journal of Pediatric Gastroenterology and Nutrition*, *49*(2), 258–261.

Jackson, K. G., Taylor, G. R., Clohessy, A. M., & Williams, C. M. (1999). The effect of the daily intake of inulin on fasting lipid, insulin and glucose concentrations in middle-aged men and women. *British Journal of Nutrition*, *82*(1), 23–30.

Jahromi, M. F., Shokryazdan, P., Idrus, Z., Ebrahimi, R., Bashokouh, F., & Liang, J. B. (2017). Modulation of immune function in rats using oligosaccharides extracted from palm kernel cake. *BioMed Research International*, *2017*, 2576921.

Kano, M., Masuoka, N., Kaga, C., Sugimoto, S., Iizuka, R., Manabe, K., et al. (2013). Consecutive intake of fermented milk containing *Bifidobacterium breve* strain Yakult and galacto-oligosaccharides benefits skin condition in healthy adult women. *Bioscience of Microbiota, Food and Health*, *32*(1), 33–39.

Kapiki, A., Costalos, C., Oikonomidou, C., Triantafyllidou, A., Loukatou, E., & Pertrohilou, V. (2007). The effect of a fructo-oligosaccharide supplemented formula on gut flora of preterm infants. *Early Human Development*, *83*(5), 335–339.

Kawakami, K., Makino, I., Asahara, T., Kato, I., & Onoue, M. (2005). Dietary galacto-oligosaccharides mixture can suppress serum phenol and p-cresol levels in rats fed tyrosine diet. *Journal of Nutritional Science and Vitaminology*, *51*(3), 182–186.

Khangwal, I., & Shukla, P. (2019). Potential prebiotics and their transmission mechanisms: Recent approaches. *Journal of Food and Drug Analysis*, *27*(3), 649–656.

Kim, G. B., Seo, Y. M., Kim, C. H., & Paik, I. K. (2011). Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. *Poultry Science*, *90*(1), 75–82.

Klatt, N. R., Canary, L. A., Sun, X., Vinton, C. L., Funderburg, N. T., Morcock, D. R., et al. (2013). Probiotic/prebiotic supplementation of antiretrovirals improves gastrointestinal immunity in SIV-infected macaques. *Journal of Clinical Investigation*, *123*(2), 903–907.

Knol, J., Boehm, G., Lidestri, M., Negretti, F., Jelinek, J., Agosti, M., et al. (2005). Increase of faecal bifidobacteria due to dietary oligosaccharides induces a reduction of clinically relevant pathogen germs in the faeces of formula-fed preterm infants. *Acta Paediatrica*, *94*(449), 31–33.

Kukkonen, K., Savilahti, E., Haahtela, T., Juntunen-Backman, K., Korpela, R., Poussa, T., et al. (2007). Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: A randomized, double-blind, placebo-controlled trial. *The Journal of Allergy and Clinical Immunology*, *119*(1), 192–198.

Langkamp-Henken, B., Bender, B. S., Gardner, E. M., Herrlinger-Garcia, K. A., Kelley, M. J., Murasko, D. M., et al. (2004). Nutritional formula enhanced immune function and reduced days of symptoms of upper respiratory tract infection in seniors. *Journal of the American Geriatrics Society*, *52*(1), 3–12.

Lee, S. I., & Kim, I. H. (2018). Difructose dianhydride improves intestinal calcium absorption, wound healing, and barrier function. *Scientific Reports*, *8*(1), 7813.

Lefranc-Millot, C., Guerin-Deremaux, L., Wils, D., Neut, C., Miller, L. E., & Saniez-Degrave, M. H. (2012). Impact of a resistant dextrin on intestinal ecology: How altering the digestive ecosystem with nutriose(r), a soluble fibre with prebiotic properties, may be beneficial for health. *Journal of International Medical Research*, *40*(1), 211–224.

Lepine, A. F. P., Konstanti, P., Borewicz, K., Resink, J. W., de Wit, N. J., Vos, P., et al. (2019). Combined dietary supplementation of long chain inulin and *Lactobacillus acidophilus* W37 supports oral vaccination efficacy against *Salmonella typhimurium* in piglets. *Scientific Reports*, *9*(1), 18017.

Liu, X., Cao, S., & Zhang, X. (2015). Modulation of gut microbiota-brain axis by probiotics, prebiotics, and diet. *Journal of Agricultural and Food Chemistry*, *63*(36), 7885–7895.

Lomax, A. R., Cheung, L. V., Tuohy, K. M., Noakes, P. S., Miles, E. A., & Calder, P. C. (2012). Beta2-1 fructans have a bifidogenic effect in healthy middle-aged human subjects but do not alter immune responses examined in the absence of an *in vivo* immune challenge: Results from a randomised controlled trial. *British Journal of Nutrition*, *108*(10), 1818–1828.

Looijer-van Langen, M. A., & Dieleman, L. A. (2009). Prebiotics in chronic intestinal inflammation. *Inflammatory Bowel Diseases*, *15*(3), 454–462.

Maguire, M., & Maguire, G. (2019). Gut dysbiosis, leaky gut, and intestinal epithelial proliferation in neurological disorders: Towards the development of a new therapeutic using amino acids, prebiotics, probiotics, and postbiotics. *Reviews in the Neurosciences*, *30*(2), 179–201.

Manning, T. S., & Gibson, G. R. (2004). Microbial-gut interactions in health and disease. Prebiotics. *Best Practice & Research: Clinical Gastroenterology*, *18*(2), 87–98.

Manning, T. S., & Gibson, G. R. (2007). Prebiotics. *Best Practice & Research: Clinical Gastroenterology*, *18*(2), 287–298.

Markowiak, P., & Slizewska, K. (2017). Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients*, *9*, 9.

Menees, S., & Chey, W. (2018). The gut microbiome and irritable bowel syndrome. *F1000Research*, *9*(7), 1029.

Messaoudi, M., Rozan, P., Nejdi, A., Hidalgo, S., & Desor, D. (2005). Behavioural and cognitive effects of oligofructose-enriched inulin in rats. *British Journal of Nutrition*, *93*(Suppl. 1), S27–S30.

Mishra, S. P., Wang, S., Nagpal, R., Miller, B., Singh, R., Taraphder, S., et al. (2019). Probiotics and prebiotics for the amelioration of type 1 diabetes: Present and future perspectives. *Microorganisms*, *7*, 3.

Miyazaki, K., Masuoka, N., Kano, M., & Iizuka, R. (2014). *Bifidobacterium* fermented milk and galacto-oligosaccharides lead to improved skin health by decreasing phenols production by gut microbiota. *Beneficial Microbes*, *5*(2), 121–128.

Moro, G., Arslanoglu, S., Stahl, B., Jelinek, J., Wahn, U., & Boehm, G. (2006). A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. *Archives of Disease in Childhood*, *91*(10), 814–819.

Mortensen, P. B., Holtug, K., & Rasmussen, H. S. (1988). Short-chain fatty acid production from mono- and disaccharides in a fecal incubation system: Implications for colonic fermentation of dietary fiber in humans. *Journal of Nutrition*, *118*(3), 321–325.

Mudd, A. T., Alexander, L. S., Berding, K., Waworuntu, R. V., Berg, B. M., Donovan, S. M., et al. (2016). Dietary prebiotics, milk fat globule membrane, and lactoferrin affects structural neurodevelopment in the young piglet. *Frontiers in Pediatrics*, *4*, 4.

Munir, M. B., Hashim, R., Nor, S. A. M., & Marsh, T. L. (2018). Effect of dietary prebiotics and probiotics on snakehead (*Channa striata*) health: Haematology and disease resistance parameters against *Aeromonas hydrophila*. *Fish and Shellfish Immunology*, *75*, 99–108.

Nagpal, R., Wang, S., Ahmadi, S., Hayes, J., Gagliano, J., Subashchandrabose, S., et al. (2018). Human-origin probiotic cocktail increases short-chain fatty acid production via modulation of mice and human gut microbiome. *Scientific Reports*, *8*(1), 12649.

Nakamura, Y. K., Janowitz, C., Metea, C., Asquith, M., Karstens, L., Rosenbaum, J. T., et al. (2017). Short chain fatty acids ameliorate immune-mediated uveitis partially by altering migration of lymphocytes from the intestine. *Scientific Reports*, *7*(1), 11745.

Nankova, B. B., Agarwal, R., MacFabe, D. F., & La Gamma, E. F. (2014). Enteric bacterial metabolites propionic and butyric acid modulate gene expression, including CREB-dependent catecholaminergic neurotransmission, in pc12 cells-Possible relevance to autism spectrum disorders. *PLoS One*, *9*(8), e103740.

Nelson, E. D., Ramberg, J. E., Best, T., & Sinnott, R. A. (2012). Neurologic effects of exogenous saccharides: A review of controlled human, animal, and in vitro studies. *Nutritional Neuroscience*, *15*(4), 149–162.

Neufeld, K. M., Kang, N., Bienenstock, J., & Foster, J. A. (2011). Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterology & Motility*, *23*(3), 255–264.

Ohta, A., Uehara, M., Sakai, K., Takasaki, M., Adlercreutz, H., Morohashi, T., et al. (2002). A combination of dietary fructooligosaccharides and isoflavone conjugates increases femoral bone mineral density and equol production in ovariectomized mice. *Journal of Nutrition*, *132*(7), 2048–2054.

Olesen, M., & Gudmand-Hoyer, E. (2000). Efficacy, safety, and tolerability of fructooligosaccharides in the treatment of irritable bowel syndrome. *The American Journal of Clinical Nutrition*, *72*(6), 1570–1575.

Ooi, L. G., & Liong, M. T. (2010). Cholesterol-lowering effects of probiotics and prebiotics: A review of in vivo and in vitro findings. *International Journal of Molecular Sciences*, *11*(6), 2499–2522.

Oyofo, B. A., DeLoach, J. R., Corrier, D. E., Norman, J. O., Ziprin, R. L., & Mollenhauer, H. H. (1989). Prevention of *Salmonella typhimurium* colonization of broilers with d-mannose. *Poultry Science*, *68*(10), 1357–1360.

Paineau, D., Payen, F., Panserieu, S., Coulombier, G., Sobaszek, A., Lartigau, I., et al. (2008). The effects of regular consumption of short-chain fructo-oligosaccharides on digestive comfort of subjects with minor functional bowel disorders. *British Journal of Nutrition*, *99*(2), 311–318.

Patel, R. M., & Denning, P. W. (2013). Therapeutic use of prebiotics, probiotics, and postbiotics to prevent necrotizing enterocolitis: What is the current evidence? *Clinics in Perinatology*, *40*(1), 11–25.

Pool-Zobel, B. L. (2005). Inulin-type fructans and reduction in colon cancer risk: Review of experimental and human data. *British Journal of Nutrition*, *93*(Suppl. 1), S73–S90.

Prasad, K. N. (1980). Butyric acid: A small fatty acid with diverse biological functions. *Life Sciences*, *27*(15), 1351–1358.

Rafter, J., Bennett, M., Caderni, G., Clune, Y., Hughes, R., Karlsson, P. C., et al. (2007). Dietary synbiotics reduce cancer risk factors in polypectomized and colon cancer patients. *The American Journal of Clinical Nutrition*, *85*(2), 488–496.

Reddy, B. S. (1998). Prevention of colon cancer by pre- and probiotics: Evidence from laboratory studies. *British Journal of Nutrition*, *80*(4), S219–S223.

Reddy, B. S., Hamid, R., & Rao, C. V. (1997). Effect of dietary oligofructose and inulin on colonic paraneoplastic aberrant crypt foci inhibition. *Carcinogenesis*, *18*(7), 1371–1374.

Revolledo, L., Ferreira, C. S., & Ferreira, A. J. (2009). Prevention of *Salmonella typhimurium* colonization and organ invasion by combination treatment in broiler chicks. *Poultry Science*, *88*(4), 734–743.

Saavedra, J., Tschernia, A., Moore, N., Abi-Hanna, A., Coletta, F., Emenhiser, C., et al. (1999). Gastro-intestinal function in infants consuming a weaning food supplemented with oligofructose, a prebiotic. *Journal of Pediatric Gastroenterology and Nutrition*, *29*(4), 513.

Savignac, H. M., Corona, G., Mills, H., Chen, L., Spencer, J. P., Tzortzis, G., et al. (2013). Prebiotic feeding elevates central brain derived neurotrophic factor, n-methyl-d-aspartate receptor subunits and d-serine. *Neurochemistry International*, *63*(8), 756–764.

Schmidt, K., Cowen, P. J., Harmer, C. J., Tzortzis, G., Errington, S., & Burnet, P. W. (2015). Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. *Psychopharmacology*, *232*(10), 1793–1801.

Sharma, S., & Puri, S. (2015). Prebiotics and lipid metabolism: A review. *Alternative Therapies in Health and Medicine*, *21*(Suppl. 3), 34–42.

Shukla, S., Shukla, A., Mehboob, S., & Guha, S. (2011). Meta-analysis: The effects of gut flora modulation using prebiotics, probiotics and synbiotics on minimal hepatic encephalopathy. *Alimentary Pharmacology & Therapeutics*, *33*(6), 662–671.

Silk, D. B., Davis, A., Vulevic, J., Tzortzis, G., & Gibson, G. R. (2009). Clinical trial: The effects of a trans-galactooligosaccharide prebiotic on faecal microbiota and symptoms in irritable bowel syndrome. *Alimentary Pharmacology & Therapeutics*, *29*(5), 508–518.

Smith, A. P. (2005). The concept of well-being: Relevance to nutrition research. *British Journal of Nutrition*, *93*(Suppl. 1), S1–S5.

Smith, A. P., Sutherland, D., & Hewlett, P. (2015). An investigation of the acute effects of oligofructose-enriched inulin on subjective wellbeing, mood and cognitive performance. *Nutrients*, *7*(11), 8887–8896.

Soleimani, N., Hoseinifar, S. H., Merrifield, D. L., Barati, M., & Abadi, Z. H. (2012). Dietary supplementation of fructooligosaccharide (FOS) improves the innate immune response, stress resistance, digestive enzyme activities and growth performance of Caspian roach (*Rutilus rutilus*) fry. *Fish and Shellfish Immunology*, *32*(2), 316–321.

Srinivasjois, R., Rao, S., & Patole, S. (2009). Prebiotic supplementation of formula in preterm neonates: A systematic review and meta-analysis of randomised controlled trials. *Clinical Nutrition*, *28*(3), 237–242.

Stinson, L. F., Payne, M. S., & Keelan, J. A. (2017). Planting the seed: Origins, composition, and postnatal health significance of the fetal gastrointestinal microbiota. *Critical Reviews in Microbiology*, *43*(3), 352–369.

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X. N., et al. (2004). Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *The Journal of Physiology*, *558*(1), 263–275.

Tahiri, M., Tressol, J. C., Arnaud, J., Bornet, F., Bouteloup-Demange, C., Feillet-Coudray, C., et al. (2001). Five-week intake of short-chain fructo-oligosaccharides increases intestinal absorption and status of magnesium in postmenopausal women. *Journal of Bone and Mineral Research*, *16*(11), 2152–2160.

Thorburn, A. N., Macia, L., & Mackay, C. R. (2014). Diet, metabolites, and western-lifestyle inflammatory diseases. *Immunity*, *40*(6), 833–842.

Tiwari, U., & Cummins, E. (2011). Meta-analysis of the effect of beta-glucan intake on blood cholesterol and glucose levels. *Nutrition*, *27*(10), 1008–1016.

Trompette, A., Gollwitzer, E. S., Yadava, K., Sichelstiel, A. K., Sprenger, N., Ngom-Bru, C., et al. (2014). Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nature Medicine*, *20*(2), 159–166.

Tschernia, A., Moore, N., Abi-Hanna, A., Yolken, R., Coletta, F., Emenhiser, C., et al. (1999). Effects of long-term consumption of a weaning food supplemented with oligofructose, a prebiotic, on general infant health status. *Journal of Pediatric Gastroenterology and Nutrition*, *29*, 503.

Van Loo, J. (2004). The specificity of the interaction with intestinal bacterial fermentation by prebiotics determines their physiological efficacy. *Nutrition Research Reviews*, *17*(1), 89–98.

Vogt, J. A., Ishii-Schrade, K. B., Pencharz, P. B., Jones, P. J., & Wolever, T. M. (2006). L-rhamnose and lactulose decrease serum triacylglycerols and their rates of synthesis, but do not affect serum cholesterol concentrations in men. *Journal of Nutrition*, *136*(8), 2160–2166.

Vulevic, J., Drakoularakou, A., Yaqoob, P., Tzortzis, G., & Gibson, G. R. (2008). Modulation of the Fecal Microflora Profile and Immune Function by a Novel Trans-Galactooligosaccharide Mixture (B-GOS) in Healthy Elderly Volunteers. *The American Journal of Clinical Nutrition*, *88*(5), 1438–1446.

Vulevic, J., Juric, A., Walton, G. E., Claus, S. P., Tzortzis, G., Toward, R. E., et al. (2015). Influence of galacto-oligosaccharide mixture (B-GOS) on gut microbiota, immune parameters and metabonomics in elderly persons. *British Journal of Nutrition*, *114*(4), 586–595.

Vulevic, J., Juric, A., Tzortzis, G., & Gibson, G. R. (2013). A mixture of trans-galactooligosaccharides reduces markers of metabolic syndrome and modulates the fecal microbiota and immune function of overweight adults. *Journal of Nutrition*, *143*(3), 324–331.

Weber, F. L., Jr. (1996). Lactulose and combination therapy of hepatic encephalopathy: The role of the intestinal microflora. *Digestive Diseases*, *14*(1), 53–63.

Whelan, K. (2013). Mechanisms and effectiveness of prebiotics in modifying the gastrointestinal microbiota for the management of digestive disorders. *Proceedings of the Nutrition Society*, *72*(3), 288–298.

Williams, S., Chen, L., Savignac, H. M., Tzortzis, G., Anthony, D. C., & Burnet, P. W. (2016). Neonatal prebiotic (BGOS) supplementation increases the levels of synaptophysin, GluN2A-subunits and BDNF proteins in the adult rat hippocampus. *Synapse*, *70*(3), 121–124.

Wilson, B., & Whelan, K. (2017). Prebiotic inulin-type fructans and galacto-oligosaccharides: Definition, specificity, function, and application in gastrointestinal disorders. *Journal of Gastroenterology & Hepatology*, *32*(Suppl. 1), 64–68.

Wolever, T. M., Spadafora, P. J., Cunnane, S. C., & Pencharz, P. B. (1995). Propionate inhibits incorporation of colonic [1,2-13c]acetate into plasma lipids in humans. *The American Journal of Clinical Nutrition*, *61*(6), 1241–1247.

Yadav, R., Singh, P. K., Puniya, A. K., & Shukla, P. (2016). Catalytic interactions and molecular docking of bile salt hydrolase (BSH) from *L. plantarum* RYPR1 and its prebiotic utilization. *Frontiers in Microbiology*, *7*, 2116.

Zhou, Z., Zhang, Y., Zheng, P., Chen, X., & Yang, Y. (2013). Starch structure modulates metabolic activity and gut microbiota profile. *Anaerobe*, *24*, 71–78.

Chapter 10

Synbiotics in Nutrition

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1 Introduction

Nowadays, besides the basic role of nutrition consisting in the supply of necessary nutrients for the growth and development of the organism, some additional aspects are becoming increasingly important, including the maintenance of health and counteracting diseases. In the world of highly processed food, particular attention is drawn to the composition and safety of consumed products. The introduction of probiotics, prebiotics, or synbiotics into the human diet is favorable for the intestinal microbiota. They may be consumed in the form of raw vegetables and fruit, fermented pickles, or dairy products. Another source may be pharmaceutical formulas and functional food. Probiotics are live microorganisms that have health benefits when consumed. Prebiotics may be used as an alternative to probiotics or as additional support for them. However, different prebiotics will stimulate the growth of different indigenous gut bacteria. Prebiotics has enormous potential for modifying the gut microbiota, but these modifications occur at the level of individual strains and species and are not easily predicted a priori. There are many reports on the beneficial effects of prebiotics on human health. High potential is attributed to the simultaneous use of probiotics and prebiotics.

The term "synbiotic" describes a combination of synergistically acting probiotics and prebiotics (Gibson & Roberfroid, 1995). A selected component introduced to the gastrointestinal tract should selectively stimulate growth and/or activate the metabolism of a physiological intestinal microbiota, thus conferring beneficial effect to the host's health. As the word "synbiotic" implies synergy, the term should be reserved for those products in which a prebiotic component selectively favors a probiotic microorganism (Cencic & Chingwaru, 2010). Synbiotics have both probiotic and prebiotic properties and were created in order to overcome some possible difficulties in the survival of probiotics in the gastrointestinal tract. Therefore an appropriate combination of both components in a single product should ensure a superior effect, compared to the activity of the probiotic or prebiotic alone. While developing a synbiotic product, the most important aspect that has taken into the account is the selection of an appropriate probiotic and prebiotic that can act separately on the host's health. The prebiotic compounds should selectively stimulate the growth of probiotics, with a beneficial effect on human health and not to be able to stimulate the other microorganisms (Markowiak & Śliżewska, 2018).

2 Synbiotics

Synbiotics are used not only for the improved survival of beneficial microorganisms added to food or feed, but also for the stimulation of the proliferation of specific native bacterial strains present in the gastrointestinal tract (Gourbeyre, Denery, & Bodinier, 2011). The effect of synbiotics on metabolic health remains unclear. It should be mentioned that the health effect of synbiotics is probably associated with the individual combination of a probiotic and prebiotic (de Vrese & Schrezenmeir, 2008). Considering a huge number of possible combinations, the application of synbiotics for the modulation of intestinal microbiota in humans seems promising (Scavuzzi, Henrique, Miglioranza, Simão, & Dichi, 2014).

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00010-1
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TABLE 10.1 Few notable prebiotics and synbiotics employed as nutritional diet in humans.

Human nutrition	
Prebiotics	**Synbiotics**
Inulin	*Lactobacillus* genus bacteria + inulin
Xylooligosaccharides	*Lactobacillus*, *Streptococcus*, and *Bifidobacterium* genus bacteria + fructooligosaccharides
Lactitol	*Lactobacillus*, *Bifidobacterium*, *Enterococcus* genus bacteria + fructooligosaccharides
Lactosucrose	*Lactobacillus* and *Bifidobacterium* genus bacteria + oligofructose
Lactulose	*Lactobacillus* and *Bifidobacterium* genus bacteria + inulin

3 Synbiotic selection criteria

The first aspect to be taken into account when composing a synbiotic formula should be a selection of an appropriate probiotic and prebiotic, exerting a positive effect on the host's health when used separately. The determination of specific properties to be possessed by a prebiotic to have a favorable effect on the probiotic seems to be the most appropriate approach. A prebiotic should selectively stimulate the growth of microorganisms, having a beneficial effect on health, with simultaneous absent (or limited) stimulation of other microorganisms.

4 Synbiotics in use

A combination of *Bifidobacterium* or *Lactobacillus* genus bacteria with fructooligosaccharides in synbiotic products seems to be the most popular. Table 10.1 presents the most commonly used combinations of probiotics and prebiotics.

5 Mechanism of action of synbiotics

Considering the fact that a probiotic is essentially active in the small and large intestine and the effect of a prebiotic is observed mainly in the large intestine, the combination of the two may have a synergistic effect (Hamasalim, 2016). Prebiotics are used mostly as a selective medium for the growth of a probiotic strain, fermentation, and intestinal passage. There are indications in the literature that, due to the use of prebiotics, probiotics microorganisms acquire higher tolerance to environmental conditions, including oxygenation, pH, and temperature, in the intestine of a particular organism (Sekhon & Jairath, 2010). However, the mechanism of action of an extra energy source that provides higher tolerance to these factors is not sufficiently explained. That combination of components leads to the creation of viable microbiological dietary supplements, and ensuring an appropriate environment allows a positive impact on the host's health. The following two modes of synbiotic action are known (Manigandan, Mangaiyarkarasi, Hemaltha, Hemaltha, & Murali, 2012):

1. action through the improved viability of probiotic microorganisms;
2. action through the provision of specific health effects.

The stimulation of probiotics with prebiotics results in the modulation of the metabolic activity in the intestine with the maintenance of the intestinal biostructure, development of beneficial microbiota, and inhibition of potential pathogens present in the gastrointestinal tract (de Vrese & Schrezenmeir, 2008). Synbiotics result in reduced concentrations of undesirable metabolites, as well as the inactivation of nitrosamines and carcinogenic substances. Their use leads to a significant increase in levels of short-chain fatty acids (SCFA), ketones, carbon disulfides, and methyl acetates, which potentially results in a positive effect on the host's health (Manigandan et al., 2012). As for their therapeutic efficacy, the desirable properties of synbiotics include antibacterial, anticancerogenic, and antiallergic effects. They also counteract decay processes in the intestine and prevent constipation and diarrhea. It turns out that synbiotics may be highly efficient in the prevention of osteoporosis, reduction of blood fat and sugar levels, regulation of the immunological system, and treatment of brain disorders associated with abnormal hepatic function (Pandey, Naik, & Babu, 2015). The concept of mechanisms of synbiotic action, based on the modification of intestinal microbiota with probiotic microorganisms and appropriately selected prebiotics as their substrates, is presented in Fig. 10.1.

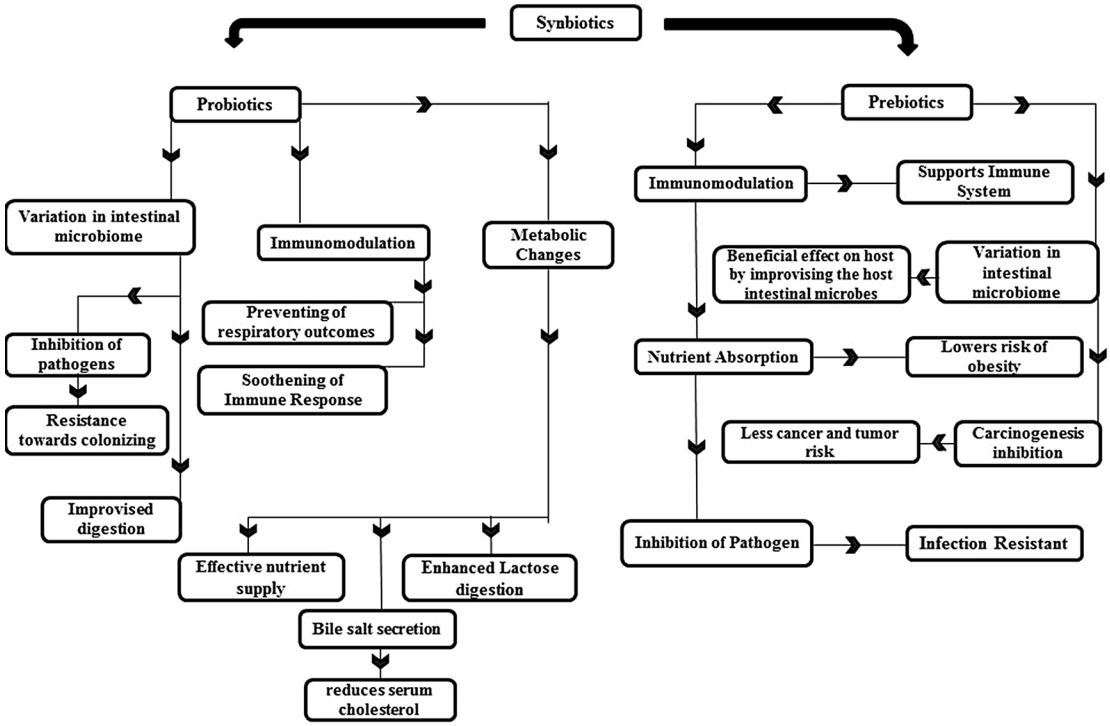


FIGURE 10.1 Effect of synbiotics and its mechanism of action.

6 Synbiotics for humans

Synbiotics role in humans is vast and has more beneficial effects (Zhang et al., 2010), namely, improved hepatic function in patients suffering from cirrhosis, increased *Lactobacillus* and *Bifidobacterium* genus count and maintenance of the balance of the intestinal microbiota, improved immunomodulatory abilities, prevention of bacterial translocation, and reduced incidence of nosocomial infections in patients' postsurgical procedures and similar interventions.

The translocation of bacterial metabolism products, such as lipopolysaccharides (LPSs), ethanol, and SCFAs, leads to their penetration of the liver. SCFAs also stimulate the synthesis and storage of hepatic triacylglycerols. Those processes may intensify the mechanisms of hepatic detoxication, which may result in hepatic storage of triacylglycerol, and intensify steatosis of the organ. A randomized trial on the use of a synbiotic containing five probiotics (*Lactobacillus plantarum*, *Lactobacillus delbrueckii* spp. *bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*) and inulin as a prebiotic in adult subjects with NASH (nonalcoholic steatohepatitis) demonstrated a significant reduction of IHTG (intrahepatic triacylglycerol) within 6 months (Wong et al., 2013a). It is also known that LPSs induce proinflammatory cytokines, such as the tumor necrosis factor alpha (TNF-α), playing a crucial role in insulin resistance and inflammatory cell uptake in NAFLD (nonalcoholic fatty liver disease). In the study on the effect of the synbiotic product containing a blend of probiotics (*Lactobacillus casei*, *L. rhamnosus*, *Streptococcus thermophilus*, *Bifidobacterium breve*, *L. acidophilus*, *Bifidobacterium longum*, *L. bulgaricus*) and fructooligosaccharides, 52 adults participated for 28 weeks. It was found that supplementation with the synbiotic resulted in the inhibition of NF-κB (nuclear factor κB) and reduced production of TNF-α (Eslamparast et al., 2014a).

In rat studies an increased level of intestinal IgA was found, following the introduction of the synbiotic product containing *L. rhamnosus* and *Bifidobacterium lactis*, and inulin and oligofructose as prebiotics to the diet. Synbiotics lead to reduced blood cholesterol levels and lower blood pressure. Moreover, synbiotics are used in the treatment of hepatic conditions and improve the absorption of calcium, magnesium, and phosphorus (Pérez-Conesa, López, Abellán, & Ros, 2006).

A metaanalysis evaluated published studies on pro/prebiotics for eczema prevention, investigating bacterial strain efficacy and changes to the allergy status of the children involved. This metaanalysis found that probiotics or synbiotics may reduce the incidence of eczema in infants aged <2 years. Systemic sensitization did not change following probiotic administration (Danq et al., 2013).

Studies carried out within the framework of the SYNCAN project funded by the European Union verified the anticarcinogenic properties of synbiotics. The effect of fructooligosaccharides (SYN1) combined with two probiotic strains

[*L. rhamnosus* GG (LGG®) and *Bifidobacterium animalis* subsp. *lactis* Bb12] on the health of patients at risk of colorectal cancer was studied. As a result, a change of biomarkers (genotoxicity, labeling index, labeled cells/crypt, transepithelial resistance, necrosis, interleukin 2, interferon) indicating the development of the disease in cancer patients, and in patients post polyp excision, was observed (Rafter et al., 2007). It was concluded that the application of the studied synbiotic may reduce the risk of colorectal carcinoma. A lower level of DNA damage was also observed, as well as a lower colonocyte proliferation ratio.

7 Synbiotics and their outcomes on human health in clinical studies

The recent human trials that applied synbiotics to treat specific clinical disorders are metabolic syndrome, inflammatory bowel disease (IBD), diarrhea, colon cancer, irritable bowel syndrome (IBS), and kidney and liver diseases. Only randomized and placebo-controlled trials (RCTs) using clearly described products are discussed.

8 Metabolic syndrome

Metabolic syndrome refers to metabolic aberrancies strongly associated with obesity that collectively contribute to the risk of coronary heart disease and type II diabetes (Cornier et al., 2008). Several synbiotic formulations have been used in clinical trials to assess the improvement of metabolic syndrome. Eslamparast et al. (2014b) conducted a double-blind RCT analyzing the effect of FOSs (250 mg) and a probiotic cocktail (Protexin®) of seven different strains (*L. casei*, *L. rhamnosus*, *S. thermophilus*, *B. breve*, *L. acidophilus*, *B. longum*, and *L. bulgaricus*) on 38 subjects suffering from metabolic syndrome. Subjects were instructed to consume the supplement or placebo (maltodextrin) twice daily for 28 weeks. They were also instructed to follow strict dietary recommendations, lower energy intake, and increase physical activity. At the end of the study, individuals in the synbiotic treatment group had improved levels of insulin resistance, fasting blood sugar, triacylglycerides, and serum high-density lipoproteins compared to the placebo treatment group. No difference was observed in body mass index, low-density lipoprotein (LDL) levels, anthropometric parameters, and energy intake/expenditure. The authors concluded that synbiotic treatment can increase the efficacy of dietary therapy in the management of metabolic syndrome and insulin resistance. However, an analysis of the gut microbiota was not included and based on the study design; it was not possible to confirm that the synbiotic treatment was more effective than the pro- or prebiotic treatment alone.

A similar synbiotic cocktail was tested for its potential ability to support a weight-loss regime (Rabiei, Shakerhosseini, & Saadat, 2015). Forty-six patients with metabolic syndrome consumed the synbiotic or placebo (maltodextrin) twice a day for 12 weeks. All patients experienced significant weight loss, and the synbiotic treatment reduced systolic blood pressure ($P < .05$). However, 90% of the subjects in the synbiotic group used medication to reduce blood pressure compared to 75% in the placebo group before and throughout the duration of the study. The fecal microbiota was not examined, and the nature of the study design precludes any conclusions about the efficacy of the pre- or probiotic strains. The combination of FOSs (3 g) with *L. plantarum*, *L. delbrueckii*, *L. acidophilus*, *L. rhamnosus*, and *B. bifidum* (2×10^8 CFU total) was assessed in an open-label RCT. The treatment was provided twice daily for 6 months to subjects suffering from NASH (Wong et al., 2013b).

A double-blind, parallel-design RCT of 20 healthy subjects found that the synbiotic treatment of FOSs (10 g) and Ecologic® 825 (1.5×10^{10} CFU) led to significantly more stools, but no differences in intestinal barrier function (measured by urinary sugar recoveries and ratios; plasma levels of zonulin, cytokines, and chemokines; and Gastro Intestinal (GI) symptom scores) were seen compared to the control group after 2 weeks of treatment (Wilms et al., 2016). Ecologic® 825 comprises *L. acidophilus*, *L. casei*, *Lactobacillus paracasei*, *L. plantarum*, *Lactobacillus salivarius*, *Lactococcus lactis*, *B. bifidum*, and two strains of *B. lactis*.

9 Inflammatory bowel disease

Ulcerative colitis (UC) and Crohn's disease (CD), the two types of IBD, are chronic inflammatory pathologies of the GI tract. Both conditions occur in individuals who are genetically susceptible and exposed to unknown environmental risk factors (Haller et al., 2010). Several synbiotic formulations have been used in clinical studies to treat IBD. A combination of 6 g of inulin/oligofructose (Synergy 1®) and 2×10^{11} CFU of *B. longum* was administered to UC patients (Furrie et al., 2005). The test strain had been isolated from a healthy human subject and assessed for its aerotolerance, acid tolerance, resistance to bile salt, and adherence to epithelial cells. Its ability to use the prebiotic substrate as an energy source was also established in vitro. The organism was further shown to alter the cytokine expression in an HT29 epithelial cell line and reduce proinflammatory cytokine levels in vitro. For the clinical study, 18 patients were divided into synbiotic and placebo

groups, each receiving the respective treatments twice daily for 4 weeks. The synbiotic treatment led to reduced inflammation and regeneration of epithelial tissue compared to the placebo group, reduced mRNA levels of human β-defensins, and lowered levels of TNF-α and IL-1α. Although survival of the probiotic strain was not measured in a strain-specific manner, bifidobacteria-specific rRNA levels increased 42 fold in the synbiotic group compared to approximately 5-fold in the placebo group. This study, however, did not investigate the effects of the probiotic independently.

In another clinical study, the effect of a synbiotic containing GOSs (5 g) and *B. breve* (1×10^9 CFU) on subjects with mild-to-moderate UC was assessed (Ishikawa et al., 2011). Forty-one patients were treated with either a placebo or the synbiotic for 1 year. Endpoints included endoscopic scores and myeloperoxidase levels in lavage solutions; both were significantly lower in the synbiotic-treated group. An analysis of the fecal microbiota by plate counting was also performed for subjects in the synbiotic group before and after the treatment. Of all assessed microbes, only Bacteroidaceae were significantly decreased after the synbiotic treatment. The abundance of *Bifidobacterium* remained the same, and *B. breve* was only detected (5.75 ± 1.65 $\log_{10}$ CFU/g feces) by culturing after the treatment. Therefore it can be concluded that *B. breve* survived passage through the GI tract. Whether or not the applied prebiotic was supporting that the probiotic could not be determined from this study. Interestingly, no bifidogenic effect was observed due to GOS treatment. This is contrary to results previously reported (Azcarate-Peril et al., 2017; Akiyama, Kimura, & Hatano, 2015; Davis, Martinez, Walter, & Hutkins, 2010).

In a double-blind RCT, patients with CD who continued using their conventional CD medication showed improvements when given a combination of Synergy 1® (6 g) and 2×10^{11} CFU *B. longum* (Steed, Macfarlane, Blackett, Bahrami, & Reynolds, 2010). Compared to a placebo, the synbiotic reduced TNF-α gene expression ($P = .041$), disease activity indexes ($P = .02$), and histological scores ($P = .018$) after 6 months of treatment. The microbiota of tissue biopsies was analyzed in both a species and a genus-specific manner. Interestingly, 8 of 13 patients in the synbiotic group had increased numbers of *B. longum* and bifidobacteria after 3 months compared to the baseline and this increased to 11 patients after 6 months. The nature of this responder/nonresponder phenomenon was not addressed in the study.

Chermesh et al. (2007) investigated the potential of Synbiotic 2000® to prevent postoperative recurrence of CD. This formulation contained *Pediococcus pentoseceus*, *Lactococcus raffinolactis*, *L. paracasei* subsp. *paracasei*, and *L. plantarum* (each at 1×10^{10} CFU) and 2.5 g each of β-glucan, inulin, pectin, and resistant starch. The frequency of the treatment was not stated. The synbiotic had no effect on endoscopic or clinical relapse or the postoperative occurrence of CD compared to the placebo. However, it significantly improved weight gain and normalization of hemoglobin levels by the 3-month follow-up. No analysis of the gut microbiota was done.

10 Diarrhea

Diarrheal diseases are often caused by infectious agents, resulting in loose or liquid bowel movements with increased frequency, water content, and volume. Worldwide, diarrhea is the leading cause of hospitalizations, morbidity, and mortality. A multistrain mixture of *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* was combined with FOSs (626 mg) as a synbiotic and was tested in children with acute diarrhea (Dinleyici et al., 2013). Treatment with oral rehydration salts (ORSs) and intravenous therapy was also provided. Compared to a control group (receiving only ORSs and/or intravenous therapy), the synbiotic shortened the duration of diarrhea ($P < .0001$) and the hospital stay ($P = .002$). The gut microbiota of these children was not analyzed.

In a similar study, children with acute rotavirus diarrhea were treated with species of *Lactobacillus*, *Streptococcus*, and *Bifidobacterium* (1×10^9 CFU total) and FOSs (990 mg). Standard fluid therapy and nutritional support were provided. Thirty-five children were enrolled in the synbiotic group and compared to a placebo group. The duration of diarrhea was significantly shorter in the synbiotic group ($P < .0001$), and for half of the patients receiving a synbiotic treatment, intestinal mucosal healing was reported 50 hours after administration. The gut microbiota was not analyzed. No further descriptions were given to explain why half of the group responded to the treatment (Dewi, Soenarto, & Karyana, 2015).

An arabinogalactan (500 mg) and xylooligosaccharide (700 mg) mixture was used to formulate a synbiotic that also included *L. paracasei* B21060 (2.5×10^9 CFU). Subjects included 55 children with acute diarrhea, who received ORS treatment. The synbiotic group showed a higher resolution rate ($P = .005$) than the placebo group after the first 72 hours (Passariello et al., 2012). This study allowed for additional treatments (e.g., diosmectite, domperidone, or racecadotril) given by the parents after the first 72 hours, which may have influenced the total duration of diarrhea. No analysis of the fecal microbiota was performed.

A combination of *S. thermophilus*, *L. bulgaricus*, *B. animalis* subsp. *lactis*, and inulin (1 g) was assessed for its potential to prevent diarrhea, vomiting, and various infections in children (Ringel-Kulka, Kotch, Jensen, Savage, & Weber, 2015). After 16 weeks of treatment, synbiotic-treated children had significantly fewer days of fever but significantly more days

with watery stools ($P < .05$). In this RCT, no analysis of the microbiota or assessment of the effect of the individual components was performed.

GI symptoms, such as diarrhea, are frequently reported among HIV and AIDS patients. The efficacy of FOSs (6 g) and a mixture of *L. paracasei*, *L. rhamnosus*, *L. acidophilus*, and *B. lactis* was tested when compared to a maltodextrin placebo. Patients ($n = 64$) consumed treatments twice daily for 6 months together with a personalized eating plan. Both the synbiotic and placebo groups had a significant reduction of diarrhea and were not significantly different from one another (da Silveira, Falco, Nery, & Turchi, 2017).

11 Irritable bowel syndrome

IBS is an intestinal disorder characterized by abdominal pain, bloating, diarrhea, constipation, or alternating periods of these symptoms. The cause of this illness has not been established, but visceral hypersensitivity, genetics, gut microbiota, constant low-grade inflammation, and environment are contributing factors (Ford et al., 2014). Approximately 11% of the world's population may be affected by IBS, with higher occurrences among women and younger individuals (Endo, Shoji, & Fukudo, 2015).

A recent study examined the effect of *L. acidophilus* (1.8×10^7 CFU/g), *B. animalis* subsp. *lactis* Bb-12 (2.5×10^7 CFU/g), and Beneo dietary fibers (2%) on the quality of life and IBS symptoms of 76 constipation-predominant IBS patients (Smid et al., 2016). The synbiotic was delivered twice daily in 180 g of fermented milk for 4 weeks. Several markers of IBS improved after 4 weeks, but there was no difference between the synbiotic and the placebo (fermented milk) groups.

An RCT assessed the effect of *L. acidophilus*, *B. animalis* subsp. *lactis*, *S. thermophilus*, and dietary fiber (90% inulin, 10% oligofructose) in fermented milk on the gut microbiota of 30 adults suffering from IBS (Matijasic et al., 2016). According to real-time polymerase chain reaction and relative afferent pupillary defect analyses of fecal samples, the presence of all three treatment bacteria was confirmed in the synbiotic group and decreased at the 1-week follow-up. There was no significant difference detected in the abundance of the test bacteria within the synbiotic treatment group; strains were not detected in the control group. The effect of the synbiotic treatment on IBS symptoms was not assessed in this study. Finally, Cudmore, Doolan, Lacey, and Shanahan (2017) showed that a synbiotic containing psyllium fiber, inulin, *L. rhamnosus*, *B. bifidum*, *L. acidophilus*, *L. plantarum*, and *L. bulgaricus* significantly reduced the use of laxatives after a 4-week treatment in adults with chronic constipation.

12 Colon cancer

Colorectal cancer is the third most common form of cancer. In addition to genetic factors, environmental factors such as radiation, chemical carcinogens, and diet contribute to tumorigenesis in the colon (Willett, 2000). Current treatments are associated with a high risk of complications and low success rates. Investigators have suggested that by maintaining a healthy weight, diet, and physical activity, up to one-third of colon cancers may be prevented (Raman et al., 2013). Numerous pro-, pre-, and synbiotic studies using rodent models suggest that these treatments may have preventive and therapeutic properties.

Rafter et al. (2007) and Roller, Clune, Collins, Rechkemmer, and Watzl (2007) assessed a combination of LGG®, *B. lactis* Bb12, and Synergy 1® on colon cancer and polypectomized patients in two similar phase II anticancer studies. The synbiotic treatment was compared to a placebo in a 12-week trial. Fecal water obtained from the cancer patients did not improve barrier function in Caco-2 cells and increased production of interferon-γ. For polypectomized patients, several benefits were observed among the synbiotic group, including decreased DNA damage in the colonic mucosa, reduced proliferation, and decreased secretion of IL-2. The investigators also assessed the survival of each of the probiotic strains in an independent study with healthy human subjects who consumed rifampicin-resistant mutants of each strain (Rafter et al., 2007). After consumption of the probiotics, both strains were isolated from feces by plating. For the study patients a fecal analysis was performed not at the strain level but at the genus level. The number of *Lactobacillus* and *Bifidobacterium* consistently increased in the synbiotic group for both cancer and polypectomized patients over the 12-week trial, whereas *Clostridium* numbers decreased. As a probiotic-only treatment was not applied, a synergy between the probiotic strains and Synergy 1® could not be confirmed on the basis of these data. Roller et al. (2007) did not analyze the fecal microbiota but referred to another study that reported that only 10% of the consumed amount of LGG® and Bb12 survived the GI tract in the same synbiotic treatment (van Loo, Clune, Bennett, & Collins, 2005). Neither study analyzed the pro- and probiotic components independently.

In a 4-week crossover trial, the effect of the synbiotic combination of resistant starch (12.5 g) and *B. lactis* (5×10^9 CFU) on markers of early colorectal carcinogenesis was investigated in 20 healthy subjects (Worthley et al., 2009). Placebo,

prebiotic-only, and probiotic-only arms were also included. Full analyses of the fecal microbiota were conducted using denaturing gradient gel electrophoresis and quantitative real-time polymerase chain reaction to assess levels of *B. lactis*. Results showed that the synbiotic treatment introduced significantly more changes to the gut microbiota than the placebo or the pro- or prebiotic treatments alone. However, levels of *B. lactis* in the probiotic and synbiotic treatment groups were not significantly different. No differences were detected for the SCFA profile, fecal ammonia or pH, serum inflammatory markers, or epithelial variables among the treatment groups.

13 Kidney and liver disease

Approximately 6–10% of adults suffer from varying degrees of chronic kidney disease (Jha et al., 2013), and the prevalence of NAFLD ranges between 6.3 and 33% (Charytoniuk, Drygalski, Konstantynowicz-Nowicka, Berk, & Chabowski, 2017). Both pro- and prebiotics have been suggested as possible therapies for these conditions (Koppe, Mafra, & Fouque, 2015). Recently, several synbiotic clinical trials for kidney and liver disease have been described. A synbiotic containing a nine-strain cocktail (bifidobacteria, lactobacilli, and streptococci) and an FOS/GOS mixture was given to chronic kidney disease patients (Rossi et al., 2016). Most of the measured biomarkers in this small study ($n = 31$) were unchanged by the treatment, although microbiota analyses revealed an increase in abundance of bifidobacteria and depletion of Ruminococcaceae. A combination of *B. longum* with FOSs was also used for the treatment of liver disease (Malaguarnera et al., 2012). Although improvements in immune biomarkers and liver function were observed in both studies, the microbiota was not assessed and individual components were not included as controls.

The effect of a seven-strain mix containing species of *Lactobacillus*, *Streptococcus*, and *Bifidobacterium* (2×10^8 CFU total) and FOSs (125 mg) was tested on 50 NAFLD patients (Mofidi et al., 2017). This RCT showed that the synbiotic group had a greater reduction of steatosis and fibrosis. Similarly, the same synbiotic cocktail improved the steatosis grade in an RCT involving 80 NAFLD patients but did not improve levels of alanine aminotransferase and aspartate transaminase compared to a placebo (Asgharian, Askari, Esmailzade, Feizi, & Mohammadi, 2016). The same cocktail plus vitamin E decreased the concentrations of liver transaminase and led to significant differences in total cholesterol and LDL cholesterol compared to the control groups (Ekhlasi et al., 2016).

14 Synbiotics for animals

The animal gastrointestinal tract, besides being the environment for a huge number of microorganisms, plays also a significant immunological role and constitutes the most important barrier protecting the host from toxins, pathogens, and consequences of their action, namely, inflammation. Currently available data regarding effects of synbiotic on animal health is insufficient and requires further studies. However, they clearly indicate the effective synergistic action of probiotics and prebiotics in the reduction of populations of bacterial gastrointestinal pathogens.

Recent years have seen a remarkable evolution in the development and applications of traditional and DNA-based molecular tools that are allowing the microbiologists to characterize and understand the microbial communities in unprecedented ways (Pontes, Lima-Bittencourt, Chartone-Souza, & Nascimento, 2007). Metagenomic investigations, comprising isolation of entire microbial community genomes, construction, and screening of clone libraries, enable the microbiologists to have a look at a more complete scenario of environmental microbial communities, and thus, to better understand the microbes–environment interactions (Singh, Gautam, Verma, Kumar, & Singh, 2008). Metagenomics could be a promising strategy for appraising of the synbiotic effect of the intestinal microbiota of animals.

The synergistic effect of *L. paracasei* bacteria is combined with FOS in the intestinal microbiota of piglets. The researchers observed an increase of total anaerobic and aerobic count and an increased number of beneficial *Lactobacillus* and *Bifidobacterium* genus bacteria in the group of animals fed with a synbiotic. At the same time, the *Escherichia coli*, *Enterobacteriaceae*, and *Clostridium* genus bacteria count decreased in the stool of studied piglets (Nemcová, Bomba, Gancarčíková, Herich, & Guba, 1999).

Mohnl, Acosta Aragon, Acosta Ojeda, Rodriguez Sanchez, and Pasteiner (2007) observed that the synbiotic product (Biomin® PoultryStar) had a comparable growth-stimulating potential to avilamycin (an antibiotic-based growth stimulant) in broiler chicken. Vicente et al. (2007) verified the effect of a synbiotic product containing *Lactobacillus* spp. with the addition of lactose. A total of 320 turkeys infected with Salmonella were bred, and a positive effect of the synbiotic on feed conversion and body weight gain of study animals was demonstrated. Li, Liu, and Xu (2008) assessed the effect of administration of FOS and *Bacillus subtilis* bacteria to broiler chickens. Total 720 broiler chickens were bred, and improvement of the average daily growth and of the feed conversion ratio, as well as reduced incidence of diarrhea and mortality of animals in comparison to animals treated with aureomycin (tetracycline antibiotic), were observed. During the administration

of a combination of GOS and *B. lactis* bacteria to broiler chickens during a 40-day rearing period, a significant increase of *Bifidobacterium* and *Lactobacillus* count and in the overall population of anaerobic bacteria was observed in the intestinal microbiota of the study animals. However, no effect on feed consumption and conversion, and on body weight was observed. Awad, Ghareeb, Abdel-Raheem, and Bohm (2009) studied the effect of the synbiotic product containing *Enterococcus faecium* bacteria and FOS as a prebiotic, and immunomodulating substances from marine algae (ficophytic substances) on the health of broiler chickens. Total 600 broiler chickens bred for 5 weeks were studied. A significant increase in the average daily body weight gain, carcass ratio, and the feed conversion ratio was found in comparison to control animals. However, no effect of the synbiotic on body weight gain was observed, except for the small intestine, in which a significant growth of intestinal villi was observed within the duodenum and the ileum. Based on the study on 240 broiler chickens, it was found that probiotics and prebiotics have a favorable effect on performance parameters, during some terms even superior to antibiotics used for the comparison. Moreover, it was observed that prebiotic supplementation may be helpful in the reduction of abdominal fat following 42 days of breeding. It was observed that probiotics and prebiotics may be possibly used as substitutes for antibiotic-based growth stimulants (Toghyani, Toghyani, & Tabeidian, 2011).

15 Synbiotic therapy

Synbiotic therapy is a powerful tool of modern medication practices for achieving quick recovery against a vast number of ailments. In clinical trials, synbiotics provided superior results over the individual treatment of prebiotic and probiotic substances. So these days, this is the easiest and cheapest way to modulate the gut microbiome in the favor of good health. Administration of synbiotics is helpful for individuals of all age groups under different kinds of disease conditions such as cancer, trauma, allergy, and IBD and postsurgery infections. Many synbiotic formulations are available in the market for public use, and research is continue to manufacturing more better than existing ones for superior activity. A large number of combinations of prebiotic and probiotic can be tried for making the best fusion product having the superior beneficial effects on the intestinal environment. Synbiotic therapy fastens the growth rate of useful microbes and simultaneously reduces the abundance of harmful bacteria because prebiotics are the substances that encourage the growth of probiotic microbes as the number of probiotic organisms increase the density of harmful species goes down. Bacteria of *Lactobacillus* and *Bifidobacterium* groups are the dominants in the gut microbiome and these are able to show some beneficial activities for maintaining the good host physiology (Gibson, Probert, Van Loo, Rastall, & Roberfroid, 2004). Application of probiotics leads to several advantageous functions such as the reduction in the frequency of antibiotic-associated diarrhea minimizes the side effects of chemotherapy, lessen the chances of rotavirus infection, modulation of the hormonal and immune response, detoxification of compounds, improved lactose intolerance, and hypertension, production of hypocholesterolemic substances, and biotransformation of metabolites (Saarela, Mogensen, Fonde, Mättö, & Mattila-Sandholm, 2000). The problem associated with probiotics is that their low survival during passages through Gastro Intestinal Tract (GIT) hence combination with prebiotic could protect them against the enzymatic and chemical reactions of pancreatic juice, bile juice, and intestinal fluid.

16 Application of synbiotics

Success of symbiotic formulae depends on the efficiency of both prebiotic and probiotic components used for the production of a particular synbiotic (Table 10.2). Till to date, many synbiotic combinations have been prepared and tried in research for promoting human health but only very few were successful and available in the market. In the process of synbiotic products development, Bengmark (2001) did a milestone work and in the year 1999 he started a project with his colleagues for the formulation of one of best combinations of probiotics and prebiotics.

For development of this combination, they screened more than 500 bacterial cultures, which have better ability to produce antioxidants, antiinflammatory proteins, especially antiinflammatory cytokines and more precious quality is to enhance the antiinflammatory properties of other bacteria. They tested their ability under the simulated conditions of gastric juice, that is, at low pH 2.5 for 2 hours and also after exposure to 20% bile for 1-hour period. After screening test of bacterial strains, they finalized seven bacteria for further study as probiotics and these bacteria were *Pediococcus pentosaceus*, *Leuconostoc mesenteroides*, *L. paracasei* F19, *L. paracasei*, *L. paracasei*, *L. plantarum* F5, and *L. plantarum* F26. These bacteria have remarkable characteristics for its application as synbiotics such as they can utilize inulin or amylopectin as sole carbon source, β-galactosidase producing, anti-Gram positive microbial substances, antimicrobial activity against oral pathogen *Candida* and gastric pathogen *Helicobacter pylori* and also reduce symptoms of lactose intolerance.

Further, among these seven, total four strains were finalized for the production of synbiotic and the development of Synbiotic 2000 which was the concluding point of this study. It is a mixture of four bacteria of *Lactobacillus* group

TABLE 10.2 Synbiotic formation study using probiotic and prebiotic components.

Probiotics	Prebiotics	Synbiotics
Lactobacillus genus bacteria	Inulin	*Lactobacillus* genus bacteria + inulin
Lactobacillus and *Bifidobacterium* genus bacteria	Inulin	*Lactobacillus* and *Bifidobacterium* genus bacteria + inulin
Lactobacillus and *Bifidobacterium* genus bacteria	Oligofructose	*Lactobacillus* and *Bifidobacterium* genus bacteria + oligofructose
Lactobacillus, *Bifidobacterium*, *Enterococcus* genus bacteria	Fructooligosaccharides	*Lactobacillus*, *Bifidobacterium*, *Enterococcus* genus bacteria + fructooligosaccharides
Lactobacillus, *Streptococcus*, and *Bifidobacterium* genus bacteria	Fructooligosaccharides	*Lactobacillus*, *Streptococcus*, and *Bifidobacterium* genus bacteria + fructooligosaccharides
Lactobacillus acidophilus La5, *Bifidobacterium lactis* Bb-12, *Streptococcus thermophilus*, and *Lactobacillus bulgaricus*	Oligofructose	*L. acidophilus* La5, *B. lactis* Bb-12, *S. thermophilus*, and *L. bulgaricus* + oligofructose
Bifidobacteria	Fructooligosaccharides	*Bifidobacteria* + fructooligosaccharides
Lactobacilli	Lactitol (lactulose)	*Lactobacilli* + lactitol
Bifidobacteria	Glucooligosaccharides	*Bifidobacteria* + glucooligosaccharides
L. acidophilus P106	Oat	*L. acidophilus* P106 + oat
Lactobacillus sp.	Rice bran derivatives	Rice bran derivatives
Lactobacillus paracasei sp. *paracasei* (FDDVS/*Lactobacillus casei*-01 nutrish, Chr. Hansen, Hoersholm, Denmark)	Fructooligosaccharides	*L. paracasei* sp. *paracasei* + Fructooligosaccharides + soya milk

(*P. pentosaceus*, *L. mesenteroides*, *L. paracasei* subsp. *paracasei*, and *L. plantarum*) along with its prebiotic component which contains four fermentable fibers such as 2.5 g of each beta-glucan, inulin, pectin, and resistant starch (Medipharm AB, Kagerod, Sweden, and Des Moines, IA). Recently, Synbiotic 15, a variant of Synbiotic 2000, is also launched and it contains three bacterial strains (*L. plantarum*, *L. paracasei*, and *Pediococcus*) instead of four bacterial strains as in Synbiotic 2000.

Synbiotic 2000 and its variant are proved very useful for those who suffer from obesity and chronic diseases. Another example of commercial synbiotic is SB3-Synbiotic marketed by the US company PuoriApS. This is constituted by using strains of *Bifidobacterium* BB-12 and *Lactobacillus fermentum* PCC along with vitamin C for boosting of the immune system. Protexin company also released a series of synbiotics, for example, Lepicol (psyllium husk, inulin, and 5 strains of live bacteria), Bio-Kult Infantis (7 strains of probiotics, high in omega-3 and vitamin D3, FOS, and gum acacia as prebiotic), multistrain Bio-Kult (14 strains of probiotics, soya, and traces of milk), Bio-Kult Pro-Cyan (cranberry extract, probiotics, and vitamin A), and Bio-KultCandéa (7 strains of probiotics, garlic, and grapefruit seed extract). More details of these products were obtained from a web server.

In the synthesis of a novel synbiotic, rice bran derivatives like RiSolubles, RiceMucil, and Cea100 are used as prebiotic with *Lactobacilli* species as probiotic (Cheruvanky, Cherukuri, Pecha, & Drell, 2009). Formulation of "Synbiotic Yoghurt" has been achieved by combining the *L. acidophilus* P106 with oats and testing of this product on mice revealed that it is a safe and beneficial product (Mahrous, El-Kholy, & Elsanhoty, 2014). Synbiotic-fortified soya milk showed a more useful effect on the gut microbiome and for production of this product, prebiotic FOS/inulin, and probiotic *L. paracasei* sp.

Usually, synbiotics developed constitute one or more probiotic bacteria along with a prebiotic component but research has been on the rise to expand the utility of synbiotics by incorporation of unique prebiotic ingredients which may provide additional effects like antioxidant activity.

17 Commercial synbiotics: obstacles, challenges, and future prospects

Synbiotic products are commonly delivered to consumers in food form, including a range of cultured dairy and nondairy products. Although prebiotics can be used in most food applications, the environmental sensitivity of probiotics (and synbiotics) may limit their practical use in many nonrefrigerated foods (Saulnier, 2008). However, new microencapsulation technologies that protect the bacteria against otherwise detrimental processing treatments could lead to a variety of new

synbiotic products, including desserts, confections, juices, cheeses, or chocolate (Ahmadi et al., 2014; Konar, Toker, Oba, & Sagdic, 2016). Interestingly, many pre- and synbiotic products contain small amounts of the probiotic component (on a per-serving basis), which may be too low to induce a health effect. Low doses are used, in part, to avoid adverse GI complaints (de Vrese & Schrezenmeir, 2008) but perhaps also for reasons of cost.

By 2018 the global market for probiotics is predicted to exceed $40 billion (Buriti, Bedan, & Saad, 2016), and the prebiotic market may soon reach $5 billion (Belorkar & Gupta, 2016). Despite these market opportunities, however, several scientific and industrial barriers exist (van den Nieuwboer, van de Burgwal, & Claassen, 2016). The development of clinically effective synbiotic combinations remains a challenging issue, and several requirements must be satisfied. As noted earlier, determining the minimum effective doses of each component is usually expected. The inclusion of suitable controls for synbiotic trials is particularly challenging. Apart from standard placebo controls, probiotic-only and prebiotic-only controls may also be necessary to confirm additive or synergistic effects. The rationale for how the pro- and prebiotic were selected and combined should also be stated. Importantly, demonstrating causality requires specific detection, quantification, and enrichment of the test strain in the GI tract. Changes introduced to the gut microbiota should also be assessed to determine whether cross-feeding or other ecological events occurred, for example, niche competition, niche partitioning, or niche exclusion with the resident microbiota.

Currently, neither the EFSA nor the FDA has approved any health claim made for pro- or synbiotic combinations. Moreover, such products must be distinguished as either a pharmaceutical product or a food product (Govender et al., 2014). The FDA guidelines state that if an agent, including probiotics, is ingested for the purpose of curing, mitigating, treating, diagnosing, or preventing disease, it is classified as a drug and must undergo the same regulatory process as any new pharmaceutical. The European Union Nutrition and Health Claims regulations intend to (1) ensure that claims are "clear, accurate and based on scientific evidence" and (2) prohibit foods that bear "claims that could mislead consumers." Ultimately, high-quality human-intervention studies are necessary to support any health claims made for a product (van Loveren, San, & Salmine, 2012).

18 The safety issue of synbiotics

The well-documented and studied synbiotic is synbiotic 2000. Till date, almost after 10 years of research on the synbiotic, it is highlighted that it comes under safe category and its consumption up to 3 months displayed no side effects. Even there is no side effect reported when its administration was done in a critically ill patient, such as HIV infected, liver transplanted, postsurgery, and patient with UC (Jain, McNaugh, Anderson, MacFie, & Mitchell, 2004). Actually, both the counterparts of synbiotic either *Lactobacillus* sp. or FOS are approved by FDA under GRAS (generally recognized as safe) category, so automatically the side effects of synbiotic became low. Although regularized research is required to observe each and every aspect related to the safety issue because probiotic are microbes and there are more chances of mutations so it is necessary to track their probiotic efficiency in each round of formulations.

19 Conclusion

For living a healthy life the use of synbiotics became popular and the need for daily life routine. In order to maintain balance in the microbial population of the intestinal lumen, it is necessary to supplement the food with additional probiotics and dietary fibers/prebiotics. It provides immunity against a wide range of infections and allergies. Simultaneously, it is very cheap and it is a natural way to enrich the most diverse and functional part of digestive tract like gut by beneficial microbiota. Any foodstuff that is considered as prebiotic and each strain of probiotic bacteria have very unique mechanism of action in GIT to provide beneficial symptoms to host health. There is a long time of research which is fully dedicated to this aspect, but still we are on the periphery of the scientific investigations which is needed for its full understanding of interactions between prebiotic and probiotic to improve the host health. Although a limited number of synbiotic formulations are available in the market as product and also in reach of public domain and a large number of products are in the trail but still, meaningful research efforts are required to improve the efficacy of the synbiotic formulations.

References

Ahmadi, A., Milani, E., Madadlou, A., Mortazavi, S. A., Mokarram, R. R., & Salarbashi, D. (2014). Synbiotic yogurt-ice cream produced via incorporation of microencapsulated *Lactobacillus acidophilus* (la-5) and fructooligosaccharide. *Journal of Food Science and Technology*, *51*(8), 1568–1574.

Akiyama, T., Kimura, K., & Hatano, H. (2015). Diverse galactooligosaccharides consumption by bifidobacteria: Implications of β-galactosidase—LacS operon. *Bioscience, Biotechnology, and Biochemistry*, *79*(4), 664–672.

Asgharian, A., Askari, G., Esmailzade, A., Feizi, A., & Mohammadi, V. (2016). The effect of symbiotic supplementation on liver enzymes, C-reactive protein and ultrasound findings in patients with non-alcoholic fatty liver disease: A clinical trial. *International Journal of Preventive Medicine*, *7*, 59.

Awad, W. A., Ghareeb, K., Abdel-Raheem, S., & Bohm, J. (2009). Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. *Poultry Science*, *88*(1), 49–56.

Azcarate-Peril, M. A., Ritter, A. J., Savaiano, A., Monteagudo-Mera, D., Anderson, C., Magness, S. T., et al. (2017). Impact of short-chain galactooligosaccharides on the gut microbiome of lactose-intolerant individuals. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(3), E367–E375.

Belorkar, S., & Gupta, A. (2016). In *Oligosaccharides: A boon from nature's desk* (pp. 658–670). (6:82). United Kingdom: AMB Express.

Bengmark, S. (2001). Pre-, pro-and synbiotics. *Current Opinion in Clinical Nutrition & Metabolic Care*, *4*, 571–579.

Buriti, F. C. A., Bedan, R., & Saad, S. M. I. (2016). Probiotic and prebiotic dairy desserts. In R. R. Watson, & V. R. Preedy (Eds.), *Cambridge, probiotics, prebiotics, and synbiotics: Bioactive foods in health promotion* (pp. 345–360). Cambridge, MA: Academic Press.

Charytoniuk, T., Drygalski, K., Konstantynowicz-Nowicka, K., Berk, K., & Chabowski, A. (2017). Alternative treatment methods attenuate the development of NAFLD: A review of resveratrol molecular mechanisms and clinical trials. *Nutrition*, *34*, 108–117.

Cencic, A., & Chingwaru, W. (2010). The role of functional foods, nutraceuticals, and food supplements in intestinal health. *Nutrients*, *2*, 611–625.

Chermesh, I., Tamir, A., Reshef, R., Chowers, Y., Suissa, A., Katz, D., et al. (2007). Failure of Synbiotic 2000 to prevent postoperative recurrence of Crohn's disease. *Digestive Diseases and Sciences*, *52*(2), 385–389.

Cheruvanky, R., Cherukuri, R., Pecha, R., & Drell, E. (2009). *Synbiotics*. . EP 1796473 A4. United States patent application, US 11/243,361.

Cornier, M. A., Dabelea, D., Hernandez, T. L., Lindstrom, R. C., Steig, A. J., Stob, N. R., et al. (2008). The metabolic syndrome. *Endocrine Reviews*, *29*(7), 777–822.

Cudmore, S., Doolan, A., Lacey, S., & Shanahan, F. (2017). A randomised, double-blind, placebo-controlled clinical study: The effects of a synbiotic, Lepicol, in adults with chronic, functional constipation. *International Journal of Food Sciences and Nutrition*, *68*(3), 366–377.

Danq, D., Zhou, W., Lun, Z. J., Mu, X., Wanq, D. X., & Wu, H. (2013). Meta-analysis of probiotics and/or prebiotics for the prevention of eczema. *Journal of International Medical Research*, *41*, 1426–1436.

Davis, L. M. G., Martinez, I., Walter, J., & Hutkins, R. (2010). A dose dependent impact of prebiotic galactooligosaccharides on the intestinal microbiota of healthy adults. *International Journal of Food Microbiology*, *144*(2), 285–292.

da Silveira, E. A., Falco, M. O., Nery, M. W., & Turchi, M. D. (2017). Effectiveness of nutritional treatment and synbiotic use on gastrointestinal symptoms reduction in HIV-infected patients: Randomized clinical trial. *Clinical Nutrition*, *36*(3), 680–685.

Dewi, M. R., Soenarto, Y., & Karyana, I. P. G. (2015). Efficacy of synbiotic treatment in children with acute rotavirus diarrhea. *Paediatrica Indonesiana*, *55*(2), 74–78.

de Vrese, J., & Schrezenmeir, M. (2008). Probiotics, prebiotics, and synbiotics. *Advances in Biochemical Engineering/Biotechnology*, *111*, 1–66.

Dinleyici, E. C., Dalgic, N., Guven, S., Ozen, M., Kara, A., Arica, V., et al. (2013). The effect of a multispecies synbiotic mixture on the duration of diarrhea and length of hospital stay in children with acute diarrhea in Turkey: Single blinded randomized study. *European Journal of Pediatrics*, *172*(4), 459–464.

Ekhlasi, G., Mohammadi, R. K., Agah, S., Zarrati, M., Hossein, A. F., Arabshahi, S. S. S., et al. (2016). Do symbiotic and vitamin E supplementation have favorite effects in nonalcoholic fatty liver disease? A randomized, double-blind, placebo-controlled trial. *Journal of Research in Medical Sciences*, *21*, 106.

Endo, Y., Shoji, T., & Fukudo, S. (2015). Epidemiology of irritable bowel syndrome. *Annals of Gastroenterology*, *28*, 158–159.

Eslamparast, T., Poustchi, H., Zamani, F., Sharafkhah, M., Malekzadeh, R., & Hetmatdoost, A. (2014a). Synbiotic supplementation in nonalcoholic fatty liver disease: A randomized, double-blind, placebo-controlled pilot study. *The American Journal of Clinical Nutrition*, *99*, 535–542.

Eslamparast, T., Zamani, F., Hekmatdoost, A., Sharafkhah, M., Eghtesad, S., Malekzadeh, R., et al. (2014b). Effects of synbiotic supplementation on insulin resistance in subjects with the metabolic syndrome: A randomised, double-blind, placebo-controlled pilot study. *British Journal of Nutrition*, *112*(3), 438–445.

Ford, A. C., Quigley, E. M., Lacy, B. E., Lembo, A. J., Saito, Y. A., Schiller, L. R., et al. (2014). Efficacy of prebiotics, probiotics, and synbiotics in irritable bowel syndrome and chronic idiopathic constipation: Systematic review and metaanalysis. *The American Journal of Gastroenterology*, *109*(10), 1547–1562.

Furrie, E., Macfarlane, S., Kennedy, A., Cummings, J. H., Walsh, S. V., O'neil, D. A., et al. (2005). Synbiotic therapy (*Bifidobacterium longum*/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: A randomised controlled pilot trial. *Gut*, *54*(2), 242–249.

Gibson, R. G., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *Journal of Nutrition*, *125*, 1401–1412.

Gibson, G. R., Probert, H. M., Van Loo, J., Rastall, R. A., & Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. *Nutrition Research Reviews*, *17*, 259–275.

Gourbeyre, P., Denery, S., & Bodinier, M. (2011). Probiotics, prebiotics, and synbiotics: Impact on the gut immune system and allergic reactions. *Journal of Leukocyte Biology*, *89*, 685–695.

Govender, M., Choonara, Y. E., Kumar, P., du Toit, L. C., van Vuuren, S., & Pillay, V. (2014). A review of the advancements in probiotic delivery: Conventional versus non-conventional formulations for intestinal flora supplementation. *AAPS PharmSciTech*, *15*(1), 29–43.

Haller, D., Antoine, J. M., Bengmark, S., Enck, P., Rijkers, G. T., & Lenoir-Wijnkoop, I. (2010). Guidance for substantiating the evidence for beneficial effects of probiotics: Probiotics in chronic inflammatory bowel disease and the functional disorder irritable bowel syndrome. *Journal of Nutrition*, *140*(3), 690S–697S.

Hamasalim, H. J. (2016). Synbiotic as feed additives relating to animal health and performance. *Advances in Microbiology*, *6*, 288–302.

Ishikawa, H., Matsumoto, S., Ohashi, Y., Imaoka, A., Setoyama, H., Umesaki, Y., et al. (2011). Beneficial effects of probiotic *Bifidobacterium* and galacto-oligosaccharide in patients with ulcerative colitis: A randomized controlled study. *Digestion*, *84*(2), 128–133.

Jain, P. K., McNaugh, C. E., Anderson, A. D., MacFie, J., & Mitchell, C. J. (2004). Influence of synbiotic containing *Lactobacillus acidophilus* La 5, *Bifidobacterium lactis* Bb 12, *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and oligofructose on gut barrier function and sepsis in critically ill patients: A randomised controlled trial. *Clinical Nutrition*, *23*, 467–475.

Jha, V., Garcia-Garcia, G., Iseki, K., Li, Z., Naicker, S., Plattner, B., et al. (2013). Chronic kidney disease: Global dimension and perspectives. *The Lancet*, *382*(9888), 260–272.

Konar, N., Toker, O. S., Oba, S., & Sagdic, O. (2016). Improving functionality of chocolate: A review on probiotic, prebiotic, and/or synbiotic characteristics. *Trends in Food Science and Technology*, *49*, 35–44.

Koppe, L., Mafra, D., & Fouque, D. (2015). Probiotics and chronic kidney disease. *Kidney International*, *88*(5), 958–966.

Li, X., Liu, L. Q., & Xu, C. L. (2008). Effects of supplementation of fructooligosaccharide and/or *Bacillus subtilis* to diets on performance and intestinal microflora in broilers. *Archiv für Tierzucht*, *51*, 64–70.

Malaguarnera, M., Vacante, M., Antic, T., Giordano, M., Chisari, G., Acquaviva, R., et al. (2012). *Bifidobacterium longum* with fructo-oligosaccharides in patients with non alcoholic steatohepatitis. *Digestive Diseases and Sciences*, *57*(2), 545–553.

Manigandan, T., Mangaiyarkarasi, S. P., Hemaltha, R., Hemaltha, V. T., & Murali, N. P. (2012). Probiotics, prebiotics and synbiotics—A review. *Biomedical and Pharmacology Journal*, *5*, 295–304.

Markowiak, P., & Śliżewska, K. (2018). The role of probiotics, prebiotics and synbiotics in animal nutrition. *Gut Pathogens*, *10*, 21.

Matijasic, B. B., Obermajer, T., Lipoglavsek, L., Sernel, T., Locatelli, I., Kos, M., et al. (2016). Effects of synbiotic fermented milk containing *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* ssp. *lactis* BB-12 on the fecal microbiota of adults with irritable bowel syndrome: A randomized double-blind, placebo-controlled trial. *Journal of Dairy Science*, *99*(7), 5008–5021.

Mofidi, F., Poustchi, H., Yari, Z., Nourinayyer, B., Merat, S., Sharafkhah, M., et al. (2017). Synbiotic supplementation in lean patients with non-alcoholic fatty liver disease: A pilot, randomised, double-blind, placebo-controlled, clinical trial. *British Journal of Nutrition*, *117*(5), 662–668.

Mahrous, H., El-Kholy, W. M., & Elsanhoty, R. M. (2014). Production of new synbiotic yoghurt with local probiotic isolate and oat and study its effect on mice. *Advances in Dairy Research*, *2*, 1–7.

Mohnl, M., Acosta Aragon, Y., Acosta Ojeda, A., Rodriguez Sanchez, B., & Pasteiner, S. (2007). Effect of synbiotic feed additive in comparison to antibiotic growth promoter on performance and health status of broilers. *Poultry Science*, *86*(1), 217.

Nemcová, R., Bomba, A., Gancarčíková, S., Herich, R., & Guba, P. (1999). Study of the effect of *Lactobacillus paracasei* and fructooligosaccharides on the faecal microflora in weanling piglets. *Berliner und Münchener tierärztliche Wochenschrift*, *112*(6–12), 225–228.

Pandey, K. R., Naik, S. R., & Babu, V. V. (2015). Probiotics, prebiotics and synbiotics—A review. *Journal of Food Science and Technology*, *52*, 7577–7587.

Passariello, A., Terrin, G., Cecere, G., Micillo, M., Marco, G., Costanzo, M. D., et al. (2012). Randomised clinical trial: Efficacy of a new synbiotic formulation containing *Lactobacillus paracasei* B21060 plus arabinogalactan and xilooligosaccharides in children with acute diarrhea. *Alimentary Pharmacology & Therapeutics*, *35*(7), 782–788.

Pérez-Conesa, D., López, G., Abellán, P., & Ros, G. (2006). Bioavailability of calcium, magnesium and phosphorus in rats fed probiotic, prebiotic and synbiotic powder follow-up infant formulas and their effect on physiological and nutritional parameters. *Journal of the Science of Food and Agriculture*, *86*, 2327–2336.

Pontes, D. S., Lima-Bittencourt, C. I., Chartone-Souza, E., & Nascimento, A. M. A. (2007). Molecular approaches: Advantages and artifacts in assessing bacterial diversity. *Journal of Industrial Microbiology and Biotechnology*, *34*, 463–473.

Rabiei, S., Shakerhosseini, R., & Saadat, N. (2015). The effects of symbiotic therapy on anthropometric measures, body composition and blood pressure in patient with metabolic syndrome: A triple blind RCT. *Medical Journal of the Islamic Republic of Iran*, *29*, 213.

Rafter, J., Bennett, M., Caderni, G., Clune, I., Hughes, R., Karlsson, P. C., et al. (2007). Dietary synbiotics reduce cancer risk factors in polypectomized and colon cancer patients. *The American Journal of Clinical Nutrition*, *85*(2), 488–496.

Raman, M., Ambalam, P., Kondepudi, K. K., Pithva, S., Kothari, C., Patel, A. T., et al. (2013). Potential of probiotics, prebiotics and synbiotics for management of colorectal cancer. *Gut Microbes*, *4*(3), 181–192.

Ringel-Kulka, T., Kotch, J. B., Jensen, E. T., Savage, E., & Weber, D. J. (2015). Randomized, double-blind, placebo controlled study of synbiotic yogurt effect on the health of children. *The Journal of Pediatrics*, *166*(6), 1475–1481.

Roller, M., Clune, Y., Collins, K., Rechkemmer, G., & Watzl, B. (2007). Consumption of prebiotic inulin enriched with oligofructose in combination with the probiotics *Lactobacillus rhamnosus* and *Bifidobacterium lactis* has minor effects on selected immune parameters in polypectomised and colon cancer patients. *British Journal of Nutrition*, *97*(4), 676–684.

Rossi, M., Johnson, D. W., Morrison, M., Pascoe, E. M., Coombes, J. S., Forbes, J. M., et al. (2016). Synbiotics easing renal failure by improving gut microbiology (SYNERGY): A randomized trial. *Clinical Journal of the American Society of Nephrology*, *11*(2), 223–231.

Saarela, M., Mogensen, G., Fonde, R., Mättö, J., & Mattila-Sandholm, T. (2000). Probiotic bacteria: Safety, functional and technological properties. *Journal of Biotechnology*, *84*, 197–215.

Saulnier, D. M. A. (2008). Synbiotics: Making the most of probiotics and prebiotics by their combinations? *Food Science & Technology Bulletin Functional Foods*, *4*, 9–19.

Sekhon, B. S., & Jairath, S. (2010). Prebiotics, probiotics and synbiotics: An overview. *Indian Journal of Pharmaceutical Education and Research*, *1*(2), 13–36.

Scavuzzi, B. M., Henrique, F. C., Miglioranza, L. H. S., Simão, A. N. C., & Dichi, I. (2014). Impact of prebiotics, probiotics and synbiotics on components of the metabolic syndrome. *Annals of Nutritional Disorders & Therapy*, *1*, 1009.

Singh, B., Gautam, S. K., Verma, V., Kumar, M., & Singh, B. (2008). Metagenomics in animal gastrointestinal ecosystem: Potential biotechnological prospects. *Anaerobe*, *14*, 138–144.

Smid, A., Strniša, L., Bajc, K., Vujić-Podlipec, D., Matijašić, B. B., & Rogel, I. (2016). Randomized clinical trial: The effect of fermented milk with the probiotic cultures *Lactobacillus acidophilus* La-5R_ and *Bifidobacterium* BB-12R_ and Beneo dietary fibres on health-related quality of life and the symptoms of irritable bowel syndrome in adult. *Journal of Functional Foods*, *24*, 549–557.

Steed, H., Macfarlane, G. T., Blackett, K. L., Bahrami, B., & Reynolds, N. (2010). Clinical trial: The microbiological and immunological effects of synbiotic consumption—A randomized double-blind placebo-controlled study in active Crohn's disease. *Alimentary Pharmacology & Therapeutics*, *32*(7), 872–883.

Toghyani, M., Toghyani, M., & Tabeidian, S. A. (2011). Effect of probiotic and probiotic as antibiotic growth promoter substitutions on productive and carcass traits of broiler chicks. In *International conference on food engineering and biotechnology* (pp. 982–986).

van den Nieuwboer, M., van de Burgwal, L. H. M., & Claassen, E. (2016). A quantitative key-opinion-leader analysis of innovation barriers in probiotic research and development: Valorisation and improving the tech transfer cycle. *Pharma and Nutrition*, *4*, 9–18.

van Loo, J., Clune, Y., Bennett, M., & Collins, J. K. (2005). The SYNCAN project: Goals, set-up, first results and settings of the human intervention study. *British Journal of Nutrition*, *93*(1), S91–S98.

van Loveren, H., San, Y., & Salmine, S. (2012). Health claims in Europe: Probiotics and prebiotics as case examples. *Annual Review of Food Science and Technology*, *3*(1), 247–261.

Vicente, J., Wolfenden, A., Torres-Rodriguez, A., Higgins, S., Tellez, G., & Hargis, B. (2007). Effect of a Lactobacillus species-based probiotic and dietary lactose prebiotic on turkey poultry performance with or without Salmonella enteritidis challenge. *J. Appl. Poult. Res*, *16*(3), 361–364.

Wong, V. W., Won, G. L., Chim, A. M., Chu, W. C., Yeung, D. K., Li, K. C., et al. (2013a). Treatment of nonalcoholic steatohepatitis with probiotics. A proof-of-concept study. *Annals of Hepatology*, *12*, 256–262.

Wong, V. W. S., Won, G. L., Chim, A. M., Chu, W. C., Wai Yeung, D. K., To Li, K. C., et al. (2013b). Treatment of nonalcoholic steatohepatitis with probiotics. A proof-of-concept study. *Annals of Hepatology*, *12*(2), 256–262.

Worthley, D. L., Le Leu, R. K., Whitehall, V. L., Conlon, M., Christophersen, C., Belobrajdic, D., et al. (2009). A human, double-blind, placebo-controlled, crossover trial of prebiotic, probiotic, and synbiotic supplementation: Effects on luminal, inflammatory, epigenetic, and epithelial biomarkers of colorectal cancer. *The American Journal of Clinical Nutrition*, *90*(3), 578–586.

Willett, W. C. (2000). Diet and cancer. *Oncologist*, *5*, 393–404.

Wilms, E., Gerritsen, J., Smidt, H., Besseling-van der Vaart, I., Rijkers, G. T., Garcia Fuentes, A. R., et al. (2016). Effects of supplementation of the synbiotic EcologicR_ 825/FOS P6 on intestinal barrier function in healthy humans: A randomized controlled trial. *PLoS One*, *11*(12), e0167775.

Zhang, M. M., Cheng, J. Q., Lu, Y. R., Yi, Z. H., Yang, P., & Wu, X. T. (2010). Use of pre-, pro-and synbiotics in patients with acute pancreatitis: A meta-analysis. *World Journal of Gastroenterology*, *16*, 3970.

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Chapter 11

Role of Probiotic Microbes Exerting Nutritional Properties

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1 Introduction

Probiotics emerged as a popular approach for the management of digestive and immune health status of human system and are being recommended as an effectual food supplement along with therapeutic measure by medical professionals. The word "probiotic" is derived from the Latin (*pro)* and Greek (*bio*), factually meaning "for life." In 1892 Albert Doderlein proposed the beneficial association between microbes and the human, he further hypothesized that the vaginal bacteria produce lactic acid that prevents the multiplication of harmful bacteria (Doderlein, 1892).

Metchnikoff, known as father of probiotics in the beginning of the 20th century itself, who was the one, earliest conceptualizer of probiotics, in 1908, reported that there are several kinds of bacteria present in the fermented milk products that produce acids, if consumed habitually, lead to healthier and long life. The probiotic Gram-positive bacteria, *Lactobacillus bulgaricus*, discovered by Metchnikoff were involved in the combination of fermented milk. There are lot of research work have been carried out by various countries in chronological years, ultimately the International Scientific Association for Probiotics and Prebiotics defines "probiotics" as "live microorganisms that, when administered in adequate amounts confer a health benefit on the host" (Hill et al., 2014).

In the beginning of the 20th century, the science of probiotics has sophisticated considerably, researchers enclose a harmony depiction, explore to recognize numerous mechanisms of action, divergent characteristics important for probiotic function, and have derived experimental substantiation supporting probiotic health reimbursement along with product excellence. Probiotics are sort of being alive and valuable microorganisms that inhabit in the gastrointestinal tract of human and rodent and as well obviously found in the fermented milk commodities. The probiotic relationship between consumption of probiotic and amelioration of metabolic problems has been entrenched by diverse studies. The microbes generally used as probiotic are LAB. The mainstay genera of microorganisms used in probiotic products are *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, *Streptococcus*, *Enterococcus*, *Escherichia*, and *Bacillus* (Fig. 11.1). Moreover, effects of probiotic has been reported to be strain dependent, although plethora of studies are imminent throughout the world on health benefits of probiotics, still there is confusion about specific and accurate way by which probiotic influence the metabolism in general disorder. Therefore, probiotics bacteria improve health by different mechanism.

Probiotics perk up the intestinal microbial equilibrium through lowering of pH to afford an argumentative environment for pathogens. Yeasts act as probiotic, to get better nutrient absorption/assimilation from food and digestion. LAB used as probiotic stimulates and balance the immune system (Raghuwanshi, Misra, Sharma, & Bisen, 2015). They aid in digestion of lactose and dairy products by reducing lactose intolerance (De Vrese, Stegelmann, Richter, Fenselau, & Laue, 2001; Almeida, Kim, Stoita, McIver, & Kurtovic, 2008), inhibit the growth of bacteria which produce nitrates in bowel in certain cases of cancer (Raghuwanshi et al., 2015), in addition, help in the regulation of bowel movements (Sullivan & Nord, 2005), prevent excessive growth of pathogenic microbes such as *Candida*, *Escherichia coli*, *Helicobacter pylori*, and *Salmonella* (Peres, Hernandez Mendonza, Bronze, Peres, & Xavier Malcata, 2015; Wu, Zhu, Chen, Chen, & Zhang, 2016; Mojgani, Fatimah, & Vaseji, 2015; Angmo, Kumari, & Bhalla, 2016; Oh & Jung, 2015), it prevents and used to treat side effects of antibiotic therapy (Ouwehand & Tennila, 2016), also reduce the toxic stack of liver (Chavez Tapia, Rodríguez, Min Seung, Lopez Ramirez, & Barbero Becerra, 2015; Vaikunthanathan, Safinia, Lombardi, & Lechler, 2016), increase the ability to absorb calcium (Katharina, Adolphi, Rochat, Barclay, & De vrese, 2016; Parvaneh, Jamaluddin, Karimi, & Erfani, 2014), reduce the occurrence of bladder cancer (Chapman, Gibson, & Rowland, 2014), therapeutic measure for

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00011-3
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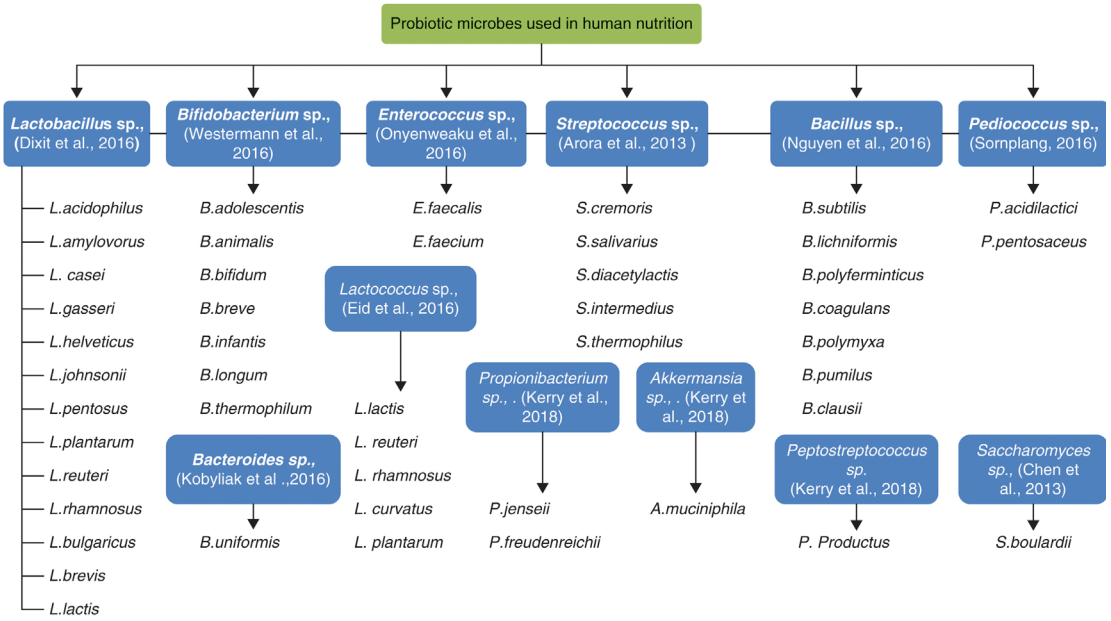


FIGURE 11.1 Various types of microbial species as probiotics.

upper respiratory complaints (De Araujo, De Oliveira, Peixoto, & Cavalcanti, 2015), increase ability to synthesize vitamin B (Li and Gu, 2016), act as remedy for bad breath (halitosis) (Bosch, Nart, Audivert, Bonachera, & Alemany, 2012), and prevent and manage atopic dermatitis (eczema) in children (Isolauri, Rautava, & Salminen, 2012). Evidences promise to be an alternative to milk, having high level of the enzyme, lactase (β-galactosidase) (Rosado, 1996), calm down the colon irritation following surgery, support healthy skin in youth, and are the primary bacteria in infants, which help them to grow and develop their immune system (Di Gioia, Aloisio, Mazzola, & Biavati, 2014; Iebba, Nicoletti, & Schippa, 2012), prevent vaginal and urinary tract infections (Reid & Devillard, 2004; Ziyadi, Bastani, Homayouni, Charandabi, & Mallah, 2016), reduce the incidence of yeast infections, virginities, and candidacies (Martinez, Franceschini, Patta, Quintana, & Candido, 2009).

Probiotics possess important functional attributes that could fulfill most of our basic nutritional and clinical supplementation requirements. There is necessitate for additional research to further clarify the most efficient probiotics for given health outcomes and to identify people most likely to respond to specific probiotic interventions. This chapter provides current perspectives on the role of probiotic microbes; in addition, it discusses evidence-based nutritional properties, considers fermented foods with regard to probiotics, and addresses a number of research challenges in front of the probiotic field.

2 Overview about probiotic foods

Probiotics are active microbial cultures that can be formulated into various types of products such as foods, dietary supplements, and drugs. The term "probiotic" is relatively used to name the microbes that are associated with the beneficial aspects for both humans and animals. These are used in fermented food products for centuries. In the current scenario, probiotics have claimed much attention due to their duel function as a food supplement and also exert as numerous health benefits. Some of these are well documented, while others shown promising potential in animal studies, further require claims in human studies. Numerous research studies documented for the probiotic effects on a various health aspects of human beings are shown in Table 11.1.

In the ninth edition of the European Pharmacopoeia (2019), probiotics defined as "live bio therapeutic products" for human use are medicinal products containing live microbes that can be administered orally or vaginally in different pharmaceutical forms (Council of Europe, 2016). New terminologies "paraprobiotic" or "postprobiotic" are emerging area in which nonviable microbial cells, their metabolites, or cell debris may contribute physiological benefits to the host by rendering added bioactivities (Pandey, Naik, & Vakil, 2015). At the same space, "prebiotics" are the substrates that are exclusively used by the host microbes and provide a health benefit (Gibson et al., 2017), and "synbiotics" are the dietary supplements of food ingredients which bring out the combinational effects of both probiotics and prebiotics (Schrezenmeir & de Vrese, 2001).

TABLE 11.1 The putative mechanisms of probiotics.

S. no.	Category	Implications	Proposed mechanisms	References
1	Management in digestive system	Irritable, bowel syndrome, symptoms affecting the GI (constipation, nonpathogenic diarrhoea, distension, flatulence, cramp, halitosis of a digestive cause)	Change in populations of the intestinal native microflora	Pace, Pace, and Quartarone, (2015)
		Lactose intolerance	Release of microbial lactase to the small intestine	Gayathri & Vasudha, 2018
2	Health protection	Allergy (atopical eczema, allergy to the milk, rheumatoid arthritis)	Translocation, barrier effect	Goel & Rishi, 2019
		Cariogenicity	Changes in the populations or activity of the oral microflora and its ability to adhere to the teeth	Elgamily, Mosallam, El-Sayed, and Mosallam (2018)
		Carcinogenicity, mutagenicity, tumor	Assimilation of the mutagen, stimulus of the immune system, reticence of carcinogen assembly by the intestinal microflora	Bhuvan and Saroj (2018)
		Diarrhoea linked to antibiotics, caused by rotavirus, colitis caused by *Clostridium difficile* and nosocomial diarrhoea	Aggressive exclusion, translocation/barrier effect, increased immune response	Guarino, Guandalini, and Lo Vecchio (2015)
		Helicobacter pylori	Antipathogenic activity	Ji and Yang (2020)
		Immunomodulation (immune status, vaccinal response)	Interaction with the immune cells or cell receptors leading to an increase in the phagocytic activity of the white cells, increasing IgA levels after exposure to the antigen, increasing the proliferation of the intraepithelial leukocytes, regulating the Th1/Th2 ratio, induction of cytokine synthesis	Yahfoufi, Mallet, Graham, and Matar (2018)
		Intestinal inflammation, ulcerative colitis, Crohn's disease, pouchitis	Immune response down regulated	Damaskos and Kolios (2008)
		Excessive intestine bacterial growth	Antimicrobial activity, competitive exclusion	Harper, Naghibi, and Garcha (2018)
		Vaginosis, urinary infections	Antipathogenic activity, competitive exclusion	Heidari, Abbaszadeh, and Mirak (2017)
3	Miscellaneous	Lowering of blood cholesterol	Deconjugation of the bile salts	Wang et al. (2018)
		Endotoxemia combined with cirrhosis	Inhibition of the production of endotoxins by the intestinal microflora	La Barz et al. (2015)
		Hypertension	Cellular constituents or peptides derived from fermentation acting as inhibitors of ACE (angiotensin-converting enzyme)	Borse, Singh, Upadhyay, Sharma, and Nivsrkar (2018)
		Renal calculi	Changes in the digestive flora influencing the breakdown of oxalate	Lieske (2017)

In the present scenario, there is an increase in the knowledge of functional foods which has focused to develop foods with health benefits in addition to adequate nutrition. The presence of probiotics in commercial food products (Table 11.2) exerts for numerous health benefits. This has led a bath to industries focusing on different applications of probiotics in food products and creating a new generation of "probiotic health" foods. This chapter focused to summarize the important application of probiotics in various food products and their function as an effective nutritive enrichment value.

TABLE 11.2 List of representative probiotic food products of various regions.

S. no.	Type of probiotic foods	Ingredients	Microbes involved	Country	References
I	**Dairy Products**				
1	Cheese	Cow milk/ buffalo milk	*Lactococcus lactis*, *Lactobacillus casei*, *Leuconostoc* spp.	Globally	Quigley et al. (2011)
2	Kefir	Sheep, goat/cow milk	*Lactobacillus brevis*, *Lactobacillus bulgaricus*, *Saccharomyces cerevisiae*	Russia	Rosa et al. (2017)
3	Borhani	Cow milk/ buffalo milk	*Lactobacillus acidophilus*, *Lactobacillus lactis*	Bangladesh	Nahidul-Islam, Kuda, Takahashi, and Kimura (2018)
4	Rubbing milk cake	Goat milk	*Lactobacillus* sp., *Lactococcus* sp.	China	Liu, Kuda, Takahashi, and Kimura (2018a)
5	Yogurt	Animal milk	*Streptococcus salivarius* subsp. *thermophilus*, *L. acidophilus*, *Bifidobacterium*	America, Australia, Europe	Angelakis, Million, Henry, and Raoult (2011)
II	**Nondairy products**				
	Vegetables				
1	Kimchi	Cabbage	*Leuconostoc mesenteroides*, *Lactobacillus plantarum*	Korea	Hongu et al. (2017)
2	Burong, mustasa, dak guadong	Mustard leaves	*L. brevis*, *L. plantarum*	Philippines, Thailand	Panghal et al. (2018)
3	Kanji	Carrot and beetroot	Lactic acid bacteria	India	Lamba, Goomer, and Saxena (2019)
4	Yan-cai	Mixed vegetables	*Lactobacillus*, *Leucobacter*	China	Liu et al. (2018b)
5	Ca muoi	Egg plant	Lactic acid bacteria	Vietnam	La Anh (2015)
	Fruits				
1	Olive oil	Olive	*L. plantarum*, *L. mesenteroides*, *L. brevis*	Spain, Italy	Sherahi, Shahidi, Tabatabaee, and Hashemi (2018)
2	Hardaliye	Red grape	*L. plantarum*	Turkey	Kilic, Agdas, Karahan, and Cakmakci (2016)
3	Pobuzihi	Cummingcordia	*Lactobacillus pobuzihii*	Taiwan	Swain, Anandharaj, Ray, and Rani (2014)
	Cereals				
1	Adai	Cereals, legumes	*Pediococcus* sp., *Streptococcus* sp., *Leuconostoc* sp.	India	Ozyurt and Otles (2014)
2	Aya-bisbaya	Rice	Lactic acid bacteria	Mexico	Champagne (1996)
3	Burukutu	Sorghum, cassava	Lactic acid bacteria, *S. cerevisiae*	Nigeria	Sridevi, Halami, and Vijayendra, (2010)
4	Idly, dosa	Rice and black gram	*Lactobacillus delbrueckii*, *S. cerevisiae*, *Lactobacillus fermenti*	India, Sri lanka, Malaysia, Singapore, and Nepal	Sridevi et al. (2010)

TABLE 11.2 List of representative probiotic food products of various regions. (*Cont.*)

S. no.	Type of probiotic foods	Ingredients	Microbes involved	Country	References
5	Jalebi	Wheat flour	*L. fermenti, L. lactis*	India, Pakistan, and Nepal	Asgar and Chauhan (2019)
6	Sour dough	Rye, wheat	*L. delbrueckii, L. plantarum, L. casei*	Australia, America, and Europe	Pontonia & Rizzello, 2019
	Meat				
1	Alheira	Pork/beef	*L. plantarum, L. brevis*	Portugal	Campos et al. (2013)
2	Pastirma	Beef/ Buffalo	*L. plantarum, L. brevis*	Turkey	Aksu, Erdemir, and Cakici (2016)
3	Salami	Pork/ostrich	*Lactobacillus* sp.	Europe	Aquilanti, Garofalo, Osimani, and Clementi (2016)
	Fish				
1	Ngari/shidal	Fish	*L. plantarum, Lactobacillus coryniformis*	India (North eastern)	Majumdar, Roy, Bejjanki, & Bhaskar, 2016
2	Hentak	Fish	*Lactic acid bacteria*	India (North eastern)	Majumdar, Roy, Bejjanki, & Bhaskar, 2016

3 Probiotic food products

The probiotic foods exploration has risen more rapidly worldwide due to higher utilization of various populations and its awareness about their impact on health benefits. In the present scenario, these products occupied between 60% and 70% of the total functional food sale in the market (Tripathi & Giri, 2014). According to the report of Buriti, de Souza, & Saad (2016), the global market for probiotic foods and drinks was 24.8 billion euro in 2011, in 2015 over 31.1 million euro, and it was estimated to reach 43 million euro by 2020. The probiotic foods can be classified into different types based on its origin shown in Fig. 11.2. The increased consumption of probiotic foods by human has gained more attention of the researchers to find out various health benefits. There are numerous probiotic food products emerged as therapeutic boon to mankind enlisted in Table 11.3.

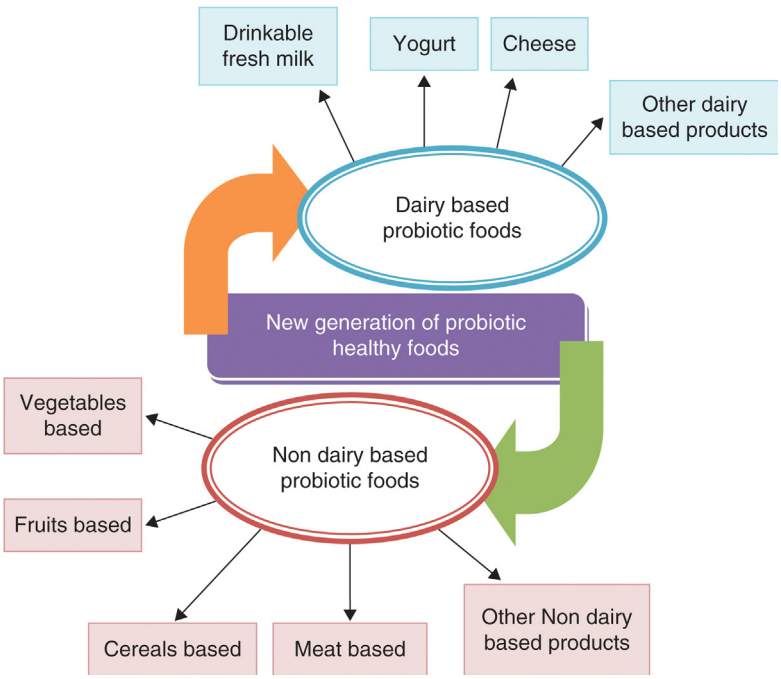


FIGURE 11.2 Probiotic foods of different categories.

TABLE 11.3 Recently popularized probiotic foods.

S. no.	Category of food	Name of the food product	References
Dairy probiotic food products			
1	**Fresh milk and fermented milk**	Yogurt	Redondo-Useros et al. (2019)
		Bioyogurt	Batawy and Khalil (2018)
		Ice cream	Goff (2008)
		Cheese	Da Cruz, Buriti, de Souza, Faria, and Saad (2009)
		Ripened cheese	Ong and Shah (2009)
		Cheddar cheese	Papanna and Agarwal (2011)
		Kefir	Ahmed et al. (2013)
		Acidophilus milk	Shiby and Misra (2013)
		Bifidus milk	Ozden (2008)
		Koumiss	Ustun (2009)
		Miru	Kilic (2008)
		Whey	Shukla and Kushwaha (2017)
Nondairy probiotic food products			
2	**Fruits and vegetables-based**	Apple	Li et al. (2019)
		Apricot juice	Bujna, Farkas, Tran, Sao Dam, and Nguyen (2018)
		Beets-based drink	Yoon et al. (2005)
		Blended orange, carrot, apple juice	Xu et al. (2019)
		Cranberry, pineapple, and orange juices	Sheehan et al. (2007)
		Cashew apple juice	Kaprasob, Kerdchoechuen, Laohakunjit, and Somboonpanyakul (2018)
		Fruit smoothies	Rodgers (2008)
		Grape and passion fruit juices	Saarela, Virkarjarvi, Alakomi, Matilla, and Matto (2006b)
		Probiotic banana puree	Tsen, Lin, and King (2009)
		Nonfermented fruit juice beverages	Renuka, Kulkarni, Vijayanand, and Prapulla (2009)
		Pineapple juice	Nguyen et al. (2019)
		Plum juice	Sheela and Suganya (2012)
		Pomegranate juice	Mantzourani et al. (2019)
		Pumpkin juice	Semjonovs et al. (2013)
		Many dried fruits	Nualkaekul, Deepika, and Charalampopoulos (2012)
		Peanut milk	Mustafa et al. (2009)
		Vegetable-based drinks	Rakin et al. (2007)
		Carrot juice and orange juice	Nazzaro, Fratinni, Sada, and Orlando (2008), Valero-Cases, Roy, Frutos, and Anderson (2017)
		Cabbage juice	Yoon, Woodams, and Hang (2006b)
		Ginger juice	Chen, Wang, and Chang (2008)
		Tomato-based drink	Liu et al. (2018a)
		Vegetable fruit beverage	Yang et al. (2018)
		Fermented banana pulp	Tsen et al. (2009)

TABLE 11.3 Recently popularized probiotic foods. (*Cont.*)

S. no.	Category of food	Name of the food product	References
3	**Cereals and soya-based**	Beverages from rice, barley, oats, wheat, soy flour	Coda, Lanera, Trani, Gobbetti, and Di Cagno (2012)
		Oat-based drink	Angelov, Gotcheva, Kuncheva, and Hrstozova (2006)
		Fermented oat flour	Duru, Kovaleva, Danilova, and Belousova (2019), Gupta and Bajaj (2017)
		Fermented beverage from maize and rice	Freire, Ramos, and Schwan (2017)
		Legume sprouts	Swieca et al. (2018)
		Cashew juice	Pereira, Maciel, and Rodrigues (2011)
		Malt-based drink	Kedia, Wang, Patel, and Pandiella (2007)
		Peanut milk	Kabeir et al. (2009)
		Maize-based substrate	Menezes et al. (2018)
		Millet-based fermented beverages	Di Stefano et al. (2017)
		Fermented soymilk drink	Donkor, Henriksson, Vasiljevic, and Shah (2007)
		Soy milk with apple juice	Içier, Gunduz, Yılmaz, and Memeli (2015)
		Wheat-based beverage	Sharma, Mridula, and Gupta (2014)
4	**Meat-based**	Fermented sausage	Bis-Souza et al. (2019b)
		Dry fermented sausage	Coelho et al. (2019), Pavli et al. (2019), Sidira, Mitropoulou, Galanis, Kanellaki, and Kourkoutas (2019)
		Beef sausage	Slima et al. (2018)
		Pork fermented sausage	Sameshima et al. (1998)
		Italian salami sausage	Ruiz, Villanueva, Favaro-Trindade, and Contreras-Castillo (2014)
5	**Chocolate-based**	Dark chocolate	Nebesny et al. (2007)
		Milk chocolate	Zaríc et al. (2016)
		Chocolate mousse	Aragon-Alegro et al. (2007)

3.1 Dairy-based probiotic foods

Dairy foods, fermented and nonfermented, impart essential roles in the food habit of human beings globally since ancient time. Milk and its derived products are best carriers of the probiotic strains because of its intrinsic properties and majority of these products are preserved in refrigerated conditions. Probiotics are found in various commercial dairy food products such as fresh and sour milk, yogurt, and cheese. They provide vital role in delivering of probiotic strains to human beings, in addition added with different health benefits. In the current situation, more than hundreds of probiotic dairy products are produced and consumed by various populations which include fermented milks, pasteurized milks, cheese, ice cream, and baby milk powders.

3.1.1 Fresh milk and fermented milk products

One of prime importance of dairy drink, milk, is consumed in larger quantities than the other probiotic beverages. Functional dairy beverages are categorized into two broad categories: fortified dairy beverages (including probiotics, prebiotics, fibers, polyphenols, peptides, aterols, minerals, vitamins, and fish oil) and whey-based beverages (Ozer & Kirmaci, 2010). *Lactobacillus rhamnosus* GG is the most commonly used probiotic strain, due to its acid and bile resistance nature, involved in the manufacturing of various dairy beverages through industrial applications. The viability of a probiotic strain in industrial processes is affected by various factors such as starter culture, media components (flavoring agents and various added additives), pH, acidity, dissolved oxygen, hydrogen peroxide, and redox potential in the fermented milks. The

different kinds of dairy beverages that contain probiotic strains are commercially available to the consumers in the market which include sweet acidophilus milk, acidophilus milk, bifidus milk, yakult, actimel, nu-trishA/B, procult drink, provivo, gaio, and others (Ozer & Kirmaci, 2010).

Probiotic strains like *Lactobacillus* and *Bifidobacterium* grow feebly in milk due to its lesser proteolytic activity and lack of ability to uptake lactose; hence, they are in need of certain growth ingredients to sustain its growth. To improve its growth pattern and viability of these strains in dairy beverages, various substances are analyzed in milk. Citrus fiber addition to the fermented milks enhances the bacterial growth and its surveillance in the product (Sendra et al., 2008). Positive effects are found out in the fermented milk with *Lactobacillus reuteri*, achieved by the addition of soy germ powder, which releases bioactive isoflavones during fermentation; in turn, it protects the strain from bile salt toxicity of the small intestine (De Boever, Wouters, & Verstraete, 2001). Other compounds such as fructo-oligosaccharides, aseinomacropeptides, whey protein concentrate, and an iron sources are also reported by Stephenie, Kebeir, Shuhaimi, Rosfarizan, & Yazid (2007).

3.1.2 Yogurt

Yogurt, traditionally and more commonly used probiotic dairy product, having numerous health benefits, is produced by the cultures of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* bacteria. Yogurt can be produced from all kind of milk-whole, low fat or fat free. The nutrients in 3.5 oz (100 g) of plain, whole-milk yogurts give 61 calories, which possess water content of 88%, 3.5 g of protein, 4.5 g of carbohydrates, and 3.3 g of fat content. Depending on the solubility in water, yogurt protein is categorized in two types as whey and casein. Water-soluble milk proteins are called whey proteins, whereas insoluble proteins are called caseins; both are rich in essential amino acids and easy to digest. Casein increases absorption of minerals like calcium and phosphorus (Holt, Carver, Ecroyd, & Thorn, 2013). Whey accounts for 20% of the protein in yogurts possess very high in branched-chain amino acids such as valine, leuine, and isoleucine. Nutritional value of the yogurts depends on the type of bacteria used in fermentation process (Tsafrakidou, Michaelidou, & Biliaderis, 2020). Vitamin B12, calcium, phosphorus, and riboflavin are the vitamins and minerals found in yogurts. Yogurt and its derived products, including low-lactose or lactose-free products and those supplemented with functional ingredients like minerals, conjugated linoleic acid, and sterols/stanols, are stands of prime importance in the market all over the world (Ortiz, Garcia-Amezquita, Acosta, & Sepulveda, 2017).

3.1.3 Cheese

Cheeses are more common dairy products posse more potential for the release of probiotics into the human intestine, due to their specific chemical and physical characteristics. They have higher pH levels, lower acidity, increased buffering capacity, more solid consistency, high fat content, increased nutrient availability, and reduced oxygen content than yogurts. These properties protect the probiotics during storage and passage through the gastrointestinal tract (Karimi, Mortazavian, & Cruz, 2011).

3.1.4 Whey

Whey or milk serum, by-product of the process of cheese production, its composition and characteristics depend on the production technology of the end product and on the quality of the milk. A good probiotic beverage with therapeutic value was prepared by using 63:35 blend ratio of whey and orange juice inoculated with probiotics of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* which extended the shelf life of the product to 45 days (Shukla & Kushwaha, 2017).

3.1.5 Other dairy products

Quark, chocolate mousse, frozen fermented dairy desserts, sour cream, and ice cream are considered to be good carriers of probiotics from the origin of dairy products. Quark was analyzed with two probiotic cultures to enhance its nutritive value, and the research findings emphasized that probiotics might assure the increased level of utilization of fat, protein, lactose, and phosphorus (Duric, Ilicic, Milanovic, Caric, & Tekic, 2007). Chocolate mousse with probiotic and prebiotic supplements was developed by Aragon-Alegro, Alegro, Cardarelli, Chiu, & Saad (2007). Reported by Wilson, Seo, Ahahbazi, & Ibrahim (2004), sour cream acts as good probiotic carrier. Ice creams have high potential probiotics carrier according to Cruz, Antunes, Sousa, Faria, & Saad (2009). The previous research findings supported the probiotic cultures not involved in the modification of sensory characteristics of ice creams, and frozen desserts also render these products bearing good viability for probiotics during its storage period.

3.2 Nondairy probiotic products

Even though dairy products are being traditionally considered as suitable source of probiotic microbes, recently, the trend of utilizing dairy products has been changed to in search of nondairy products due to lactose intolerance exists among most people. Hence, this gained the attraction of more consumers globally, focused on fruits and vegetables, cereals, chocolate, and meat- and fish-based foods of probiotic nature. There are a more number of nondairy probiotic foods and drinks available in the market in which ProVivo—first nondairy probiotic food product from Swedish company, Skane dairy in 1994, and the first nondairy probiotic drink, Good Belly—derived from oatmeal and added with *Lactobacillus plantarum* 299v in US market during 2006 (Panghal et al., 2018).

3.2.1 Fruit- and vegetable-based probiotic products

Fruits and vegetables are enriched with many essential nutritive sources and posse functional ingredients/nutritive contents such as antioxidants, vitamins, phytochemicals, minerals, and dietary fibers (Slavin & Lioyd, 2012), but some factors may restrict the viability of the probiotic such as reduced pH that is coupled with the increased levels of organic acids and dissolved oxygen (Shori, 2016). An already known fact is that fruits are healthier, energizer, and render good taste and better flavor, which is favored by all the population of various categories. Fruits and vegetables have higher nutrient and carbohydrate contents that are favored for the growth of probiotic bacteria and in combination with the fast passage via the harsh acidic environment of stomach exhibit increased probiotic cell viability (Kandylis, Pissaridi, Bekatorou, Kanellaki, & Koutinas, 2016). In contradictory to the dairy products, fruits and vegetables lack allergens, cholesterol, and lactose which exhibit negative effect to some populations.

Fruits like cashew apple, pineapple, mango, grapes, cranberry, orange, and strawberry and vegetables like carrot, beetroot, and olive are commonly consumed juices used as food substrates for probiotic bacteria deliverance. Numerous varieties of fruits and vegetables products are developed and commercialized comprising juices of fresh fruits and vegetables, fermented vegetables, dried fruits, and deserts for vegetarians (Rivera-Espinoza & Gallardo-Navarro, 2010). Probiotic juices can be prepared either by direct addition of the strain into the juice or by the fermentation of the juice with probiotic bacteria. The later process gains more importance than the former due to the fermentation of the juice with this strain; metabolites such as bacteriocin can be produced which helps for the improvement of the product and also increases the shelf life during its storage (Pereira & Rodrigues, 2018). In a research study of Rakin, Vukasinovic, Siler-Marinkovic, & Maksimovic (2007), they reported that the enrichment of beetroot and carrot juice with brewer's yeast autolysate before fermentation with *L. acidophilus* enriched it with pigments, minerals, and vitamins and also decreased the fermentation time and affected the viability of probiotic strain. The fortification of apple juice with oat flour, containing 20% of beta-glucan, could protect *L. rhamnosus* under refrigerated storage (Saarela, Virkajarvi, Nohynek, Vaari, & Matto, 2006a). For the development of novel probiotic fruit products from tomato (Yoon, Woodams, & Hang, 2004), beet juice (Yoon, Woodams, & Hang, 2005), and cabbage juice (Yoon, Woodamns, & Hang, 2006a), four strains of probiotic LAB such as *L. acidophilus*, *L. plantarum*, *Lactobacillus casei*, and *L. delbrueckii* are employed.

3.2.2 Cereals and soya-based probiotic products

All over the world, most commonly consumed food products are cereals such as maize, wheat, barley, oat, and other grains, having multifarious nutrient composition (Kumar, Vijayendra, & Reddy, 2015). They contribute substantial amount of proteins, carbohydrates, vitamins, minerals, fibers, and oligosaccharides, which enables the growth of probiotic bacteria and also healthy nondairy carriers of probiotic foods (Prado, Parada, Pandey, & Soccol, 2008). Cereals contain various dietary fibers (Knudsen, Nerskov, Bolig, Hedemann, & Laerke, 2017) and contain the main components of nonstarch polysaccharides such as arabinoxylans, beta-glucans, cellulose, resistant starch, fructans, and lignin along with small molecular weight bioactivates (phenolics and minerals). From ancient timings, fermented cereals can be used by our ancestors because these products increase the availability of minerals like phosphorus, calcium, and iron in addition with some microbial enzymes such as phytase, also some organic acids produced during the process. Nondairy cereal-based fermented beverages like boza, bushera, pozol, mahewu, and togawa are also carriers of probiotics. Biofortification in vitamins of fermented cereal products has been attempted by some researchers (Saubade, Hemery, Guyot, & Humblot, 2017). The incorporation of *Lactococcus lactis* N8 and *Saccharomyces boulardii* SAA655 in idli batter increased the riboflavin and folate content by 40%–90% (Rajendran, Chamlagain, Kariluoto, Piironen, & Saris, 2017).

Research study of Helland, Wicklund, & Narvhus (2019) reported that maize porridge can be prepared by using maize flour and barley malt along with four probiotic strains of *L. reuteri, L. acidophilus* (LA5 and 1748), and *L. rhamnosus* GG as a good probiotic food. In the research findings of Charalampopulos & Pandiella (2010), *L. plantarum* can be able to grow and viable in fermented products comprising extracts of barley, wheat bran, and malt even under refrigerated conditions.

A recent study of Ai, Li, Su, & Meng (2015) reported a novel multicereal-based fermented beverages by using the probiotic strain *Lactobacillus helveticus* KLDS1.9204. Menezes, Ramos, Dias, & Schwan (2018) reported their findings on the commercial probiotic strain *Lactobacillus paracasei* LBC-81 individually and also along with the combination of potential probiotic yeasts such as *Saccharomyces cerevisiae* CCMA0731, *S. cerevisiae* CCMA0732, and *Pichia kluyveri* CCMA0615 that are used to ferment a maize-based substrate. Recently, Lebos-Pavunc et al. (2019) reported that three novel dehydrated wheat/rice cereal-based functional products with the addition of probiotic strain *Bifidobacterium animalis* subsp. *lactis* BB-12 was developed for infants of 1–4 months.

3.2.3 Meat-based probiotic products

Meat products are an excellent carrier for the release of probiotic bacteria. Fermented sausages (dry sausages) are effective focused meat products with probiotics, which are processed without heat treatment, hence, probiotics sustain in the final product (Arihara & Ohata, 2011). The most commonly used probiotics in fermented meat products are *L. casei*, *L. paracasei*, *L. plantarum*, *L. rhamnosus*, *Lactobacillus sakei*, *Pediococcus acidilactici*, and *Pediococcus pentosaceus* in the foods of fuet, Italian salami, Swiss salami, mutton-fermented sausages, Norwegian salami, Hungarian salami, and dry cured pork loins (Aspri, Papademaas, & Tsaltas, 2020). Fermented sausages are considered as smart carriers for the growth and survival of probiotics because of the protection of the bacterial cells to reduce pH and bile salts which are exerted from the fat molecules in the passage of gastrointestinal tract and enhanced the growth of probiotics by the presence of probiotic fibers (Bis-Souza, Barba, Lorenzo, Penna, & Barretto, 2019a). Research findings of Slima et al. (2018) emphasize the effects of fermented beef sausages on microbiological and physicochemical characteristics by using a mixed culture of bioactive probiotic strains of *L. plantarum* TN8 and *P. acidilactici* MA 18/5M which prolong sausage shelf life and also improve the cooking yield and texture. In another findings, Pavli, Argyri, Chorianopoulos, Nychas, & Tassou (2019) examined the efficiency of *L. plantarum* L125 that was used as adjunct culture for the production of probiotic dry-fermented pork sausages.

3.2.4 Chocolate-based probiotic products

Chocolate is an extensively consumed product by all the populations due to its delicious taste and flavor, nutritive value, fast metabolism, and easy digestibility. Emerging trend focused toward the consumption of chocolate which not only imparts health benefit but also prevents heart disease, osteoporosis, cancer, and diabetes too. Cocoa is considered as functional food due to the presence of enriched sources of polyphenolic antioxidants, proteins, carbohydrates, minerals, and flavonoids. Plenty of research reports emphasized that chocolate is a good candidate for probiotic bacteria as the lipid fraction of cocoa butter supports protection to probiotics during storage and also in upper gastrointestinal tract passage (Kemsawasd, Chaikham, & Rattanasena, 2016). In a study, Klindt-toldam et al. (2016) reported that *L. acidophilus* NCFM and *Bifidobacterium lactis* HN019 added into chocolate (dark and milk chocolate) maintain the viability during storage and also during the passage of the upper gastrointestinal track, without harming the sensory quality of the chocolates.

Many research findings established the viability and stability of probiotics in chocolate substrates during storage. According to the study of Nebesny, Żyżelewicz, Motyl, & Libudzisz (2007), the freeze–dried *L. casei* and *L. paracasei* probiotic strains supplemented in dark chocolate maintain a stable viability in refrigerated conditions. Chocolate mousse is an admirable carrier for the supplementation of *L. paracasei* subsp. *paracasei* LBC82 alone or in combination with the probiotic substance inulin (Aragon-Alegro et al., 2007). Recent findings of Mirkovic et al. (2018) encapsulated that probiotic strain *L. plantarum* 564 and the commercial probiotic strain *L. plantarum* 299v were added in dark chocolate and brought extended shelf life of the product.

In addition, probiotics from traditional fermented foods are mainly used to produce a probiotic food drink, nutrition supplements, and feed additive to improve health in humans, animals, or plants. They have been explored in different categories depending on their enormous benefits such as production, research, environmental protection, disease control, and others. In their research finding, Zhao et al. (2019) reported that ProBiotic Data Base comprises 1730 known probiotics (768 production, 264 research, 22 environmental protection, 17 disease control, and 659 others), which provides the biological information for its use in humans, plants, and animals. This database effectively functions toward the analysis of biological information and the mechanisms of probiotics to predict novel probiotics from unknown microbial resources.

4 Microbial role in probiotic foods and nutritional properties

The health benefit of fermented foods is enhanced by supplementation of various kinds' probiotic microbial species. Probiotics exert health effects by nonspecific, species-specific, and strain-specific mechanisms.

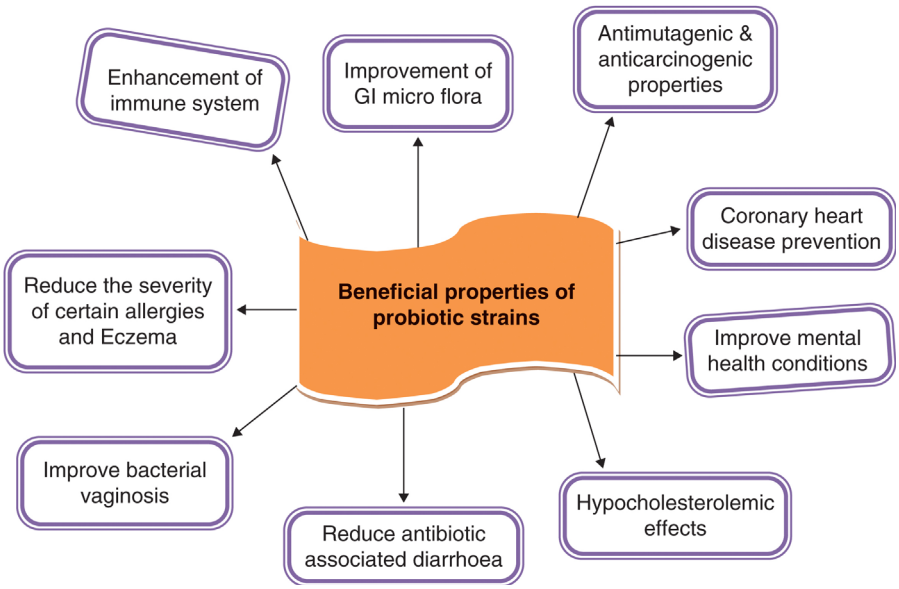


FIGURE 11.3 Recognized valuable properties of probiotic strains.

- The nonspecific mechanisms vary widely among strains, species, or even genera of commonly used probiotic supplements. These mechanisms include inhibition of the growth of pathogenic microorganisms in the gastrointestinal tract (by fostering colonization resistance, improving intestinal transit, or helping normalize a perturbed microbiota), production of bioactive metabolites (e.g., short-chain fatty acids), and reduction of luminal pH in the colon.
- Species-specific mechanisms can include vitamin synthesis, gut barrier reinforcement, bile salt metabolism, enzymatic activity, and toxin neutralization.
- Strain-specific mechanisms, which are rare and are used by only a few strains of a given species, include cytokine production, immunomodulation, and effects on the endocrine and nervous systems.

Through all of these mechanisms, probiotics might have wide-ranging impacts on human health and disease (Hill et al., 2014). Some of the recognized valuable properties of probiotic strains are shown in Fig. 11.3.

Probiotics revealed three different modes of actions against the pathogenic microbes. The prime importance is the competition for nutrients and in search for ecological niche of pathogenic flora in the digestive system. Probiotics have specific effect to other microbes by the inhibition of pathogenic adhesion, which is helpful for the maintenance of internal health condition. Some of the major probiotics, such as *Lactobacilli* and *Bifidobacteria*, were studied for their active inhibition of pathogenic colonization by their efficient antagonistic activity against gastrointestinal pathogens. The second mechanism implies that active production of potential antimicrobial substances (Fig. 11.4) such as bacteriocins, toxins, short-chain fatty acids, organic acids, and reduction in gut pH, which are involved in the destruction of food borne or spoilage causing

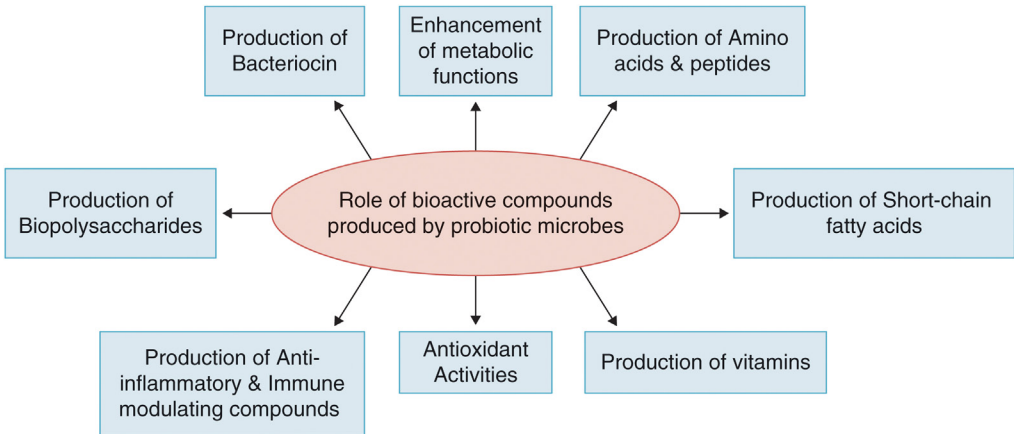


FIGURE 11.4 Importance of bioactive compounds by probiotic microbes.

pathogens in gastrointestinal environment, leads to the habitat where it could not sustain. The third mode of action explains about the stimulation/modulation of specific and nonspecific immune responses by T-cell activation, to cytokine production and IgA secretion, alterations in T-cell responses, etc. This could be an important mechanism in the prevention and treatment of infectious diseases (Maria, Dimitrios, & Stavroula, 2013; Abatenh, Gizaw, & Tsegay, 2018).

The intake of high-calorie foods and low physical activity had affected the immune system of host and increased the mortality rate (Bosma-den Boer, van Wetten, & Pruimboom, 2012). Yoo & Kim, (2016) reported that the probiotic microbes would reduce the side effects emerging from the unavoidable modern life style, by restoring the gut dysbiosis. The Indian traditional foods have a plethora of probiotic microbes, antioxidant activity, nutritive properties, dietary fibers, etc. and hence are recognized as "functional foods" (Sarkar, Lohithkumar, Dhumal, Panigrahi, & Choudhary, 2015). Recent development is focused on the intestinal survival of probiotic bacteria, their competition with the abundant resident microbiota, identification of activity, and clarification of mechanisms of action. Probiotics have to survive gastrointestinal transit and arrive viable to contribute positively to the activity of the intestinal microflora, and thus, the health of the host (Toma & Pokrotnieks, 2006).

5 Probiotic food and its clinical significance—human health perspectives

Probiotics are live microbial food supplements that provide several health benefits, as they help in maintaining excellent stability and composition of the intestinal microbiota and boost the resistance against infection by pathogens. They can be considered as potential functional foods, as they offer health benefits much more than the traditional nutritional foods. The requirement for probiotic functional foods is rapidly and progressively on the rise because of increased awareness of the public regarding the impact of food on health. Probiotics are now emerging as a promising key category of food supplement around the globe. There are numerous evidences backed up with high-quality scientific data to advocate that probiotic involvement could indeed be successful in the different types of diarrhoeal diseases, the modulation of immune function, the prevention of colon cancer, and other chronic gastrointestinal inflammatory disorders (Begum et al., 2017).

The variety of food products with probiotic strains are emerged as therapeutic boon to many kinds of diseases. The major products available in the present scenario are dairy-based which include fermented milks, cheese, ice cream, butter milk, milk powder, and yogurt that are the largest selling commodities of sales. Nondairy food products include soy-based, nutrition bars, cereals, and a wide variety of juices having probiotic delivery to the clients (Ewe, Wan Nadiah, & Liong, 2010; Sheehan, Ross, & Fitzgerald, 2004). A huge number of evidences were claimed toward the increased beneficial effects to the probiotics comprising intestinal health development, improvement in immunological responses, lowering of serum cholesterol, and anticancer properties. Recently, an increasing curiosity of consumers toward healthy food products and also the use of probiotics that provide better health benefits attract the attention of scientific community to search for the microbial management as a health promoting approach (Barros et al., 2020).

5.1 Antimicrobial potential of probiotic foods

LAB are often inhibitory to other microbes; this forms the basis of their ability to improve the keeping quality and safety of many food products. Bacteriocins are bactericidal peptides, produced by the LAB, and are extensively studied in recent years. Existence of these microbes in food regarded as "natural" and they are acceptable as food preservatives. Nisin is a type of bacteriocin having wide practical application in food industries, produced by certain strains of *L. lactis.* It differs from many other bacteriocins produced by LAB in having a relatively broad spectrum of activity against Gram-positive bacteria. Hydrogen peroxide is well known for its antimicrobial properties. Because LAB posse a number of flavoprotein oxidases but lack the degradative enzyme catalase, they produce hydrogen peroxide in the presence of oxygen. Lactic acid fermentations are essentially anaerobic processes so hydrogen peroxide formation will be limited by the amount of oxygen dissolved in the substrate at the start of fermentation. Heterofermentative LAB produce ethanol, another well-known antimicrobial agent, that often contributes to the inhibition of competitors, even though its concentration in lactic-fermented products is generally low. Fermented foods have long reputation for being positively beneficial to human health since the ordinary foods are not having these properties. A number of claims have been made for LAB, particularly in association with fermented milks, for their active presence that is termed "probiotic foods."

5.2 Antiinflammatory activity of probiotic food and human health

Many research findings emphasis that the functional aspects of probiotics are highly strain specific, and their biological effects cannot be estimated at species level (Ramos, Thorsen, Schwan, & Jespersen, 2013). This provokes the researchers

to search for novel probiotic strains with important health benefits of human beings. Many *Lactobacillus* species isolated, from dahi, kefir, and kimchi, are having Th1/Th2 immune cells by a rise in antiinflammatory cytokines like IL-12 and IFN-α (Tamang, Shin, Jung, & Chae, 2016). Catherine et al. (2018) recently reported that *Lactobacillus fermentum* MCC2760 is isolated from human faeces having antiinflammatory activity during LPS-induced $Caco_2$ cells. Devi, Kurrey, & Halami (2018) explained their findings that the selected strains exhibited a probiotic effect against LPS-challenged $Caco_2$ cells by stimulating immunomodulatory CKs and brought out important role in controlling homeostasis and host gut immunity. Further, they elucidated the potent probiotic strain *Lactobacillus paraplantarum* MTCC 9483, from vegetable origin, having strong antiinflammatory activity during oxidative stress, LPS induction, and pathogen invasion conditions, with improved levels for IL-4, IL-10, and TLR-2.

5.3 Antiobesity and probiotic foods

Probiotics and nutraceuticals have evidence that significantly lower prevalence of obesity, reduction of insulin resistance, total and VAT weight in the rat MSG obesity model with omega-3 might have most beneficial antiobesity properties (Kobyliak et al., 2018). Antiobesity effects of probiotics are reported by Jeanne et al. (2016), and its mechanism for lowering body weight (Park & Bae, 2015). Some probiotics produce short-chain fatty acids like butyrate, propionate, and acetate that are involved in the regulation of food intake and induce the satiety through gut peptides (GLP-1, PYY) (Torres-Fuentes, Schellekens, Dinan, & Cryan, 2015).

5.4 Probiotic food and antidiabetic activities

International Diabetes Federation of Southeast Asia reported that 425 million people have diabetes worldwide, including 78 million people in this region alone (International Diabetes Federation, 2017). Even though many dedications are available, no definitive cure till date. Hence, biomolecular and pharmacological researchers are trying to find out therapeutic measures to cure this disorder by using synbiotics (Iqbal et al., 2014). Larsen et al. (2010) evidenced that the enhancement of the beneficial microbiota by the use of probiotics is employed to play a significant role in neutralization of the disorder. The other convincing strategy for the management of type 2 diabetes emphasizes that the modulation of gut hormones like gastric inhibitory polypeptide and glucagon like peptide-1 may be recommended through probiotic and probiotic interventions in which hormones play an important role in homeostasis and results in neutralizing the disorder of peripheral insulin resistance or beta cells unable to secrete insulin (Grover, Rashmi, Srivastava, & Batish, 2012). Several research reports analyzed NF-κB (nuclear factor kappa B) being a promising target for diabetic treatment in which probiotics found to be as best biotherapeutic agent for the treatment of inflammatory disorders and many chronic clinical stages (Bhardwaj et al., 2020).

5.5 Anticancer properties of probiotic foods

The potential of probiotics acted as an anticarcinogenic agent to impart its ability to alter bacterial enzyme activity and also acted as antigenotoxic agent by both in vitro and in vivo studies (Burns & Rowland, 2000). The strains of *Lactobacillus* and *Bifidobacterium*, their cellular components, and metabolites showed decreased DNA damage in the colon cells. *Bifidobacterium longum* and *L. acidophilus* proved decreased activity of procarcinogenic enzyme and higher enzymatic activities of beta-glucuronidase, nitroreductase, and azoreductase (Pool-Zobel, Neudecker, Domizlaff, Ji, & Schillinger, 1996). Butyrate produced from the probiotics can decrease the proliferation of cancer cells and increase the apoptosis process (Riviere, Selak, Lantin, Leroy, & De Vuyst, 2016). Bhuvan & Saroj (2018) reported that probiotics showed anticancerous activity by producing antitumorigenic compounds, reduced the efficiency of carcinogens by binding to them, or decreased the absorption of mutagens through intestine and enhancing the immune system of the host. The immense-valued probiotics can be explored by using traditionally fermented foods that can able to improve the healthiness of human population and also protect from dreadful diseases.

5.6 Probiotic foods and its effect on brain and CNS

An effect of probiotics on the CNS has been investigated in clinical trials in which gut microbiota influence human brain development function (Tillisch, 2014). In a research study Messaoudi et al. (2011) exhibited the reduction in psychological distress in some healthy volunteers administered with *L. helveticus* R0052 and *B. longum* R0175 by taking random analyses. A regular intake of *L. plantarum* WCFSI (4.5×10^{10} CFU/day) to the children with autism spectrum disorder

can have improvements in their school records and also their food habitual (Umbrello & Esposito, 2016). In a research study, Szajewska (2016) emphasized that autism spectrum and attention deficit/hyperactivity disorders in children could be overcome by *L. rhamnosus* intake to the mother during fourth weeks from expected delivery. This was further observed that many gut bacteria are able to produce neuroactive compounds resemblance of those produced in brain. The strains derived from human intestinal origin, *Lactobacillus brevis* DPC6108 and *Bifidobacterium dentium*, were able to produce higher amount of gamma-aminobutyric acid—a brain neurotransmitter involved in the suppression of anxiety and depression (Barrett, Ross, O'Toole, Fitzgerald, & Stanton, 2012). Research findings of Nogueiras, Romero-pica, Vazquez, Novelle, & Lopez (2012) emphasized that oral administration of probiotic strain, *L. acidophilus*, helps people to regulate their mood toward rewards and addictive behaviors.

6 Role of probiotics in dietary supplements

Probiotic products for human consumption, available in the commercial aspects, are of two forms as either food products or drug (food) supplements. In food supplements, high counts of viable probiotic cells (minimum of 10^{10} CFU/mL) in the form of tablet or capsule are taken up into the body, whereas in food products, the microbes are added along with suitable carrier systems or applied as starter probiotics in fermented foods. Therefore a regulated interactions and interventions are carried out in food matrices that adversely affect the viability of initially inoculated probiotics in food systems before consumption (Meybodi & Mrtazavian, 2017).

An addition of probiotics in dietary supplements mainly utilizes them in the freeze–dried powder format such as capsules, tablets, and powder in stick packaging or sachets, which are usually preserved at room temperature. Dietary supplement products may deliver the probiotics notified on the label during the entire shelf life period of the products. This guaranteed the consumers be given the sufficient dose of probiotics to the targeted group for the assured health claim or the health benefit (Fenster et al., 2019).

Probiotics are considered as complementary and alternative medicine in addition to minerals, vitamins, and other dietary supplements (April et al., 2012). In the present scenario, they are commercially available substances present not only in dietary supplements, functional foods, drugs, and beverages but also in products like vaginal capsules, chewable tablets for gum health, skin creams, and tampons (Vecchione et al., 2018).

7 Global emergence of probiotic foods

Probiotic foods are most emerging sector all around the world, by employing the microbes as diet additives or of pharmaceuticals. Europe being the first place in terms of more number of probiotic foods are utilized, followed by Japan (Aggarwal, Nagori, & Mathur, 2012). In European countries the rate of traditional consumption of natural probiotic products such as fermented milk is in increased level. They consume probiotics in the form as foods and also foods supplements. They not only depend on dairy-based probiotics but also other nondairy products like fruit juices, berry soups, and soy-based and cereal-based fermented products. Japan is recognized as functional food origin place, where probiotics along with functional foods are more commonly used by the people (Hilliam, 2000). According to the study of Side, 2006; Fern (2007); Amagase (2008), there are 500 different products commercially available in their market.

In the United States, probiotic consumption is practiced by the population toward preventive health care. In the present scenario, the United States possesses the biggest functional foods and nutraceutical products market around the globe in which probiotic products are of major concern (Basu, Thomas, & Acharya, 2007). Canadian population generally depends on various nutraceuticals and functional food–assisted probiotics in their consumption. China could be a dominant producer of nutritional supplements all around the world. There are more than 3000 domestic manufacturers of nutritional supplements comprising 559 foreign brands and nearly 10,000 health food products on the market (Sharma, Arora, & Baldi, 2013). In India, major pharmaceutical companies focused their attention toward the production of packaged probiotic-based nutritional supplements suited for special needs like lactation, pregnancy, and immunodeficiency ranged from pediatric to geriatric population. In addition, probiotics are employed as food supplements for dairy animals such as cattle, poultry, and piggery (Balaji & Kantha, 2011). In Australia, probiotics engage a liminal space between medicine and food which are termed "therapeutic good." There are nearly 245 different probiotic products enlisted on the Australian Register of Therapeutic Goods, which are consumed by the population without prescription from the medical practitioner (Australian Register of Therapeutic Goods, 2019). In Austria and Germany, probiotics are considered as nutrients and derive the common term as dietary supplements and dietetic foods (Rechtsinformationssystem des Bundes, 2019).

8 Nutraceutical importance of probiotic foods

The term "functional food" was first introduced in Japan in the mid-1980s and it refers to "processed foods containing ingredients that aid specific bodily functions in addition to being nutritious." Toma and Pokrotnieks (2006) reported that they are considered as those foods intended to be constituted as part of a normal diet, and that contain biologically active components, which offer the potential of enhanced health or reduced risk of disease. Functional food was defined as "food or dietary components which provide a health claim in addition with its basic function of supply of nutrients" (Cencic & Chingwaru, 2010). Fermented cereal products are appropriate for the people who have lactose intolerance, milk allergies or people who follow a reduced lipid consumption or vegan dietary guide. These are considered as novel probiotic delivery vehicles and effective functional foods (Wuyts, Van Beeck, Allonsius, van den Broek, & Lebeer, 2020).

Research findings demonstrated that nutrition plays a crucial role in the prevention of chronic disease, as most of them can be related to diet. Functional foods are not only necessary for the living but also as a source of mental and physical well-being, contributing to the prevention and reducing of risk factors for several diseases or enhancing certain physiological functions. Emerging global interest focused on the functional foods containing nutrients capable of supplementing effective health benefits is gradually increasing both on interest and consumer demand (Nazhand et al., 2020). Explorative study (Durazzo et al., 2020) on the application of nanotechnology to nutraceuticals in the form of nanoprobiotics gained a new dimension of research, which imparts the nanoparticles loaded with a specific probiotic component that may enhance the absorption mechanism in the gastrointestinal tract.

9 Future perspectives of probiotic foods

Probiotics bring out numerous health credentials to the welfare of human beings. Probiotic therapies are the emerging area of interest through which we can claim therapeutic measures for the situation like infectious, allergic, inflammatory, and neoplastic. Before getting into the exploration of suitable probiotics for regular consumption, efficient evaluation of these products is mandatory. To satisfy these criteria, standards concerning quality and reliability should be analyzed. Hence, in future ingenious placebo-controlled research studies along with legalized outcomes are needed for ascertaining the important health benefits of these products. Before focusing on this area of research, vital aspects to be noted are careful selection of the probiotic strain, its dosage of standardization, and in-depth knowledge of their beneficial health effects so that, this therapy can be an efficient and attractive tool in the field of medicine for the well-being of mankind. Regardless of many research findings on the beneficial effects of probiotic on health, their effects on various diseases not been fully investigated and their mode of action for promoting health controlling disorders also a need of further investigation. In addition, the dose-dependent consequence of probiotics is a tedious issue that has to be resolved due to short-term consumption of beverages, leads a limited impact on the pathological process, and they have complex compounds, various types of active ingredients, and poor standardization.

10 Conclusion

Probiotics are emerged as vital area of numerous categories of foods that are providing more nutritional inputs to the health system. They comprise bacteria and their metabolites exerting health-enhancing properties. These are having attractive improvement in immune system, avert allergies and having a healing potential of the intestine. A superior diet, complemented with a high-quality probiotic, can improve the maintenance between harmful and harmless bacteria and also posse the nutritional and therapeutic activities. Probiotics can be extensively studied and explored commercially in many different products in the world. Many research findings emphasized that probiotics exerted more beneficial effects to the welfare of human beings. The nonpathogenic microbes are considered as probiotics comprising wide variety of species and sub species that are having the ability to adhere, colonize, and modulate the human GI system which is not a universal property. The growing global interest in probiotic foods containing nutrients capable of supporting possible beneficial health effects is rapidly increasing both interest and also the demand of consumers.

In the present scenario, there are a number of factors like higher antimicrobial resistance raised in pathogenic microbes and also amplified hassle of consumers for innate proxy for the medicines paved the path of health professionals to explore alternatives to pharmaceutical drugs. The advent and exploration of the scientific and clinical trials evidenced that the effectiveness of assured probiotic strains served as gorgeous biotherapeutics for mankind.

References

Abatenh, E., Gizaw, B., Tsegay, Z., Tefera, G., & Aynalem, E. (2018). Health benefits of probiotics. *Journal of Bacteriology and Infectious Diseases*, *2*(1), 8–27.

Aggarwal, A., Nagori, B. P., & Mathur, V. (2012). Regulatory approval of probiotics in US, Japan & India: A comparative study. *International Journal of Pharmaceutical Sciences and Research*, *2*, 78–86.

Ahmed, Z., Wang, Y., Ahmed, A., Khan, S. T., Nisa, M., Ahmed, H., et al. (2013). Kefir and health. A contemporary perspective. *Critical Reviews in Food Science and Nutrition*, *53*(5), 422–434.

Ai, J., Li, A. L., Su, B. X., & Meng, X. C. (2015). Multi-cereal beverage fermented by *Lactobacillus helveticus* and *Saccharomyces cerevisiae*. *Journal of Food Science*, *80*, M1259–M1265.

Aksu, M. I., Erdemir, E., & Cakici, N. (2016). Changes in the physico-chemical and microbial quality during the production of pastirma cured with different levels of sodium nitrite. *Korean Journal for Food Science of Animal Resources*, *36*(5), 617–625.

Almeida, J. A., Kim, R., Stoita, A., McIver, C. J., Kurtovic, J., et al. (2008). Lactose absorption in the elderly: Role of small intestinal bacterial overgrowth. *Scandinavian Journal of Gastroenterology*, *43*, 146–154.

Amagase, H. (2008). Current marketplace for probiotics: A Japanese perspective. *Clinical Infectious Diseases*, *46*, S73–S75.

Angelakis, E., Million, M., Henry, M., & Raoult, D. (2011). Rapid and accurate bacterial identification in probiotics and yogurts by MALDI-TOF mass spectrometry. *Journal of Food Science*, *76*, M568–M572.

Angelov, A., Gotcheva, V., Kuncheva, R., & Hrstozova, T. (2006). Development of a new oat-based probiotic drink. *International Journal of Food Microbiology*, *112*, 75–80.

Angmo, K., Kumari, A., & Bhalla, T. C. (2016). Probiotic characterization of lactic acid bacteria isolated from fermented foods and beverage of Ladakh. *LWT—Food Science and Technology*, *66*, 428–435.

April, K. T., Moher, D., Stinson, J., Byme, A., White, M., Boon, H., et al. (2012). Measurement properties of questionnaires assessing complementary and alternative medicine use in paediatrics. A systemic review. *PLoS One*, *7*, e39611.

Aquilanti, L., Garofalo, C., Osimani, A., & Clementi, F. (2016). Ecology of lactic acid bacteria and coagulase negative cocci in fermented dry sausages manufactured in Italy and other Mediterranean countries: An overview. *International Food Research Journal*, *3*(2), 429–445.

Aragon-Alegro, L. C., Alegro, J. H. A., Cardarelli, H. R., Chiu, M. C., & Saad, S. M. I. (2007). Potentially probiotic and synbiotic chocolate mousse. *LWT—Food Science and Technology*, *40*, 669–675.

Arihara, K., & Ohata, M. (2011). Functional meat products. *Functional foods*. Amsterdam, The Netherlands: Elsevier. pp. 512–533.

Asgar, S., & Chauhan, M. (2019). Contextualization of traditional dairy products of India by exploring multidimensional benefits of heating. *Trends in Food Science and Technology*, *88*, 243–250. doi: 10.1016/j.tifs.2019.03.033.

Aspri, M., Papademaas, P., & Tsaltas, D. (2020). Review on non-dairy probiotics and their use in non-dairy based products. *Fermentation*, *6*, 30. doi: 10.3390/fermentation6010030.

Australian Register of Therapeutic Goods. 2019. Available online: https://www.tga.gov.au/artg Accessed 02.04.19.

Balaji, R. R., & Kantha, D. A. (2011). Market potential for probiotic nutritional supplements in India. *African Journal of Business Management*, *5*, 5418–5423.

Barrett, E., Ross, R. P., O'Toole, P. W., Fitzgerald, G. F., & Stanton, C. (2012). Gamma Aminobutyric acid production by culturable bacteria from the human intestine. *Journal of Applied Microbiology*, *113*, 411–417. doi: 10.1111/j.1365-2672.2012.05344.x.

Barros, C. P., Guimaraes, J. T., Esmerino, E. A., Duarte, M. C. K. H., Silva, M. C., Silva, R., et al. (2020). Paraprobiotics and postbiotics: Concepts and potential applications in dairy products. *Current Opinion in Food Science*, *32*, 1–8. https://doi.org/10.1016/j.cofs.2019.12.003.

Basu, S. K., Thomas, J. E., & Acharya, S. N. (2007). Prospects for growth in global nutraceutical and functional food markets: A Canadian perspective. *Australian Journal of Basic and Applied Sciences*, *1*(4), 637–649. Available from.

Batawy, O. E., & Khalil, O. S. (2018). Manufacture and properties of low fat bio-yogurt containing probiotic strains and maltodextrin as probiotic. *Journal of Probiotics & Health*, *6*, 192. doi: 10.4172/2329-8901.1000192.

Begum, P. S., Madhavi, G., Rajagopal, S., Viswanath, B., Razak, M. A., & Venkataratnamma, V. (2017). Probiotics as functional foods: Potential effects on human health and its impact on neurological diseases. *International Journal of Nutrition, Pharmacology, Neurological Diseases*, *7*, 23–33. doi: 10.4103/ijnpnd.ijnpnd_90_16.

Bhardwaj, R., Singh, B. P., Sandhu, N., Singh, N., Kaur, R., Rokana, N., et al. (2020). Probiotic mediated NF-κB regulation for prospective management of type 2 diabetes. *Molecular Biology Reports*, *47*, 2301–2313. https://doi.org/10.1007/s11033-020-05254-4.

Bhuvan, S., & Saroj, A. (2018). Probiotics: A promising anti-cancer agent. *Advances in Biotechnology & Microbiology*, *10*(2), 555783. doi: 10.19080/AJBM.2018.10.555783.

Bis-Souza, C., Barba, F., Lorenzo, J., Penna, A. B., & Barretto, A. (2019a). New strategies for the development of innovative fermented meat products: A review regarding the incorporation of probiotics and dietary fibers. *Food Reviews International*, *35*, 467–484.

Bis-Souza, C. V., Pateiro, M., Domínguez, R., Lorenzo, J. M., Penna, A. L. B., & da Silva Barretto, A. C. (2019b). Volatile profile of fermented sausages with commercial probiotic strains and fructo oligosaccharides. *Journal of Food Science and Technology*, *56*, 5465–5473.

Borse, S. P., Singh, D. P., Upadhyay, D., Sharma, V., & Nivsrkar, M. A. (2018). Probiotic use in the management of hypertension: A new era of therapeutic management. *Indian Journal of Health Sciences and Biomedical Research*, *11*, 207–214. doi: 10.4103/kleuhsj.kleuhsj_3_18.

Bosch, M., Nart, J., Audivert, S., Bonachera, M. A., Alemany, A. S., Fuentes, M. C., et al. (2012). Isolation and characterization of probiotic strains for improving oral health. *Archives Oral Biology*, *57*, 539–549.

Bosma-den Boer, M. M., van Wetten, M. L., & Pruimboom, L. (2012). Chronic inflammatory diseases are stimulated by current life style: How diet, stress levels and medication prevent our body from recovering. *Nutrition & Metabolism*, *9*(32), 1–14. http://dx.doi.org/10.1186/1743-7075-9-32.

Bujna, E., Farkas, N. A., Tran, A. M., Sao Dam, M., & Nguyen, Q. D. (2018). Lactic acid fermentation of apricot juice by mono-and mixed cultures of probiotic *Lactobacillus* and *Bifidobacterium* strains. *Food Science and Biotechnology*, *27*, 547–554.

Buriti, F. C. A., de Souza, C. H. B., & Saad, S. M. I. (2016). Cheese as probiotic carrier: Technological aspects and benefits. *Handbook of animal-based fermented food and beverage technology*. Boca Raton, FL, USA: CRC Press. pp. 764–799.

Burns, A. J., & Rowland, I. R. (2000). Anti-carcinogenicity of probiotics and prebiotics. *Current Issues in Intestinal Microbiology*, *1*(1), 13–24.

Campos, S. D., Alves, R. C., Mendes, E., Costa, A. S., Casal, S., & Oliverira, M. B. (2013). Nutritional value and influence of the thermal processing on a traditional Portuguese fermented sausage (alheira). *Meat Science*, *93*(4), 914–918.

Cencic, A., & Chingwaru, W. (2010). The role of functional foods, nutraceuticals and food supplements in intestinal health. *Nutrients*, *2*, 611–625.

Champagne, C. P. (1996). Some technological challenges in the addition of probiotic bacteria to foods. In D. Charalampopoulos, & R. A. Rastall (Eds.), *Prebiotics and probiotics science and technology* (pp. 761–804). New York, NY: Springer.

Chapman, C. M. C., Gibson, G. R., & Rowland, I. (2014). Effects of single and multi-strain probiotics on biofilm formation and *in vitro* adhesion to bladder cells by urinary tract pathogens. *Anaerobe.*, *27*, 71–76.

Charalampopulos, D., & Pandiella, S. S. (2010). Survival of human derived *Lactobacillus plantarum* in fermented cereal extracts during refrigerated storage. *LWT—Food Science and Technology*, *43*, 431–435.

Chavez Tapia, N. C., Rodríguez, L. G., Min Seung, J., Lopez Ramirez, Y., Barbero Becerra, V. J., Juárez-Hernández, E., et al. (2015). Current evidence on the use of probiotics in liver diseases. *Journal of Functional Foods*, *17*, 137–151.

Chen, I. N. C. C., Wang, C. Y., & Chang, T. L. (2008). Lactic fermentation and antioxidant activity of Zingiberaceae plants in Taiwan. *International Journal of Food Sciences and Nutrition*, *22*, 1–10.

Coda, R., Lanera, A., Trani, A., Gobbetti, M., & Di Cagno, R. (2012). Yogurt-like beverages made of a mixture of cereals, soy and grape must: Microbiology, texture, nutritional and sensory properties. *International Journal of Food Microbiology*, *155*, 120–127.

Coelho, S. R., Lima, Í. A., Martins, M. L., Junior, A. A. B., de Almeida Torres Filho, R., Ramos, A. D. L. S., et al. (2019). Application of *Lactobacillus paracasei* LPC02 and lactulose as a potential symbiotic system in the manufacture of dry-fermented sausage. *LWT*, *102*, 254–259.

Council of Europe. (2016). *European pharmacopoeia* (9th ed.). Strasbourg, France: Council of Europe. p. 6522.

Cruz, A. Z., Antunes, A. E. C., Sousa, A. L. O. P., Faria, J. A. F., & Saad, S. M. I. (2009). Ice-cream as a probiotic food carrier. *Food Research International*, *42*, 1233–1239.

Da Cruz, A. G., Buriti, F. C. A., de Souza, C. H. B., Faria, J. A. F., & Saad, S. M. I. (2009). Probiotic cheese: Health benefits, technological and stability aspects. *Trends in Food Science and Technology*, *20*, 344–354.

Damaskos, D., & Kolios, G. (2008). Probiotics and prebiotics in inflammatory bowel disease: Micro flora 'on the scope'. *British Journal of Clinical Pharmacology*, *65*(4), 453–467. doi: 10.1111/j.1365-2125.2008.03096.x.

De Araujo, G. V., De Oliveira, H., Peixoto, D. M., & Cavalcanti, E. S. S. (2015). Probiotics for the treatment of upper and lower respiratory-tract infections in children: Systematic review based on randomized clinical trials. *Jornal De Pediatria*, *91*, 413–427.

De Boever, P., Wouters, R., & Verstraete, W. (2001). Combined use of *Lactobacillus reuteri* and soygerm powders as food supplement. *Letters in Applied Microbiology*, *33*, 420–424.

De Vrese, M., Stegelmann, A., Richter, B., Fenselau, S., Laue, C., & Schrezenmeir, J (2001). Probiotics compensation for lactase insufficiency. *The American Journal of Clinical Nutrition*, *73*, 421S–429S.

Devi, S. M., Kurrey, N. K., & Halami, P. M. (2018). *In vitro* anti-inflammatory activity among probiotic *Lactobacillus* species isolated from fermented foods. *Journal of Functional Foods*, *47*, 19–27. https://doi.org/10.1016/j.jff.2018.05.036.

Di Gioia, D., Aloisio, I., Mazzola, G., & Biavati, B. (2014). *Bifidobacteria*: Their impact on gut micro biota composition and their applications as probiotics in infants. *Applied Microbiology and Biotechnology*, *98*, 563–577.

Di Stefano, E., White, J., Seney, S., Hekmat, S., McDowell, T., Sumarah, M., et al. (2017). A novel millet-based probiotic fermented food for the developing world. *Nutrients*, *9*, 529.

Doderlein, A. S. G. (1892). *Das Scheidensekret und seine Bedeutung fur das Puerperalfieber* (Vol. 11). Leipzig: E. Besold. p. 699.

Donkor, O. N., Henriksson, A., Vasiljevic, T., & Shah, N. P. (2007). α-Galactosidase and proteolytic activities of selected probiotic and dairy cultures in fermented soymilk. *Food Chemistry*, *104*, 10–20.

Durazzo, A., Nazhand, A., Lucarini, M., Atanasov, A. G., Souto, E. B., Novellino, E., et al. (2020). An updated overview on nanonutraceuticals: Focus on nanoprebiotics and nanoprobiotics. *International Journal of Molecular Sciences*, *21*(7), 2285. https://doi.org/10.3390/ijms21072285.

Duric, M. S., Ilicic, M. D., Milanovic, S. D., Caric, M. D., & Tekic, M. N. (2007). Nutritive characteristics of Probiotic Quark as influenced by type of starter. *Acta Periodica Technologica*, *38*, 11–19.

Duru, K. C., Kovaleva, E., Danilova, I., & Belousova, A. (2019). Production and assessment of novel probiotic fermented oat flour enriched with isoflavones. *LWT*, *111*, 9–15.

Elgamily, H., Mosallam, O., El-Sayed, H., & Mosallam, R. (2018). Antibacterial effectiveness of probiotic-based experimental mouthwash against cariogenic pathogen: An *in vitro* study. *European Journal of Dentistry*, *12*, 7–14. doi: 10.4103/ejd.ejd253 17.

Ewe, J. A., Wan Nadiah, W. A., & Liong, M. T. (2010). Viability and growth characteristics of *Lactobacillus* in soymilk supplemented with B-Vitamins. *International Journal of Food Sciences and Nutrition*, *61*(1), 87–107.

Fenster, K., Freeburg, B., Hollard, C., Wong, C., Laursen, R. R., & Ouwehand, A. C. (2019). The production and delivery of probiotics: A review of a practical approach. *Microorganisms*, *7*, 83. doi: 10.3390/microorganisms7030083.

Fern, E. (2007). Marketing of functional foods: A point of view of the industry. In *International developments in science & health claims, ILSI international symposium on functional foods in Europe*.

Freire, A. L., Ramos, C. L., & Schwan, R. F. (2017). Effect of symbiotic interaction between a fructooligosaccharide and probiotic on the kinetic fermentation and chemical profile of maize blended rice beverages. *Food Research International*, *100*, 698–707.

Gayathri, D., & Vasudha, M. (2018). Lactose intolerance with special emphasis on probiotics for management. *EC Nutrition*, *13*(5), 332–332.

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., et al. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology*, *14*, 491–502.

Goel, C., & Rishi, N. (2019). Importance of probiotics in management of allergic diseases – Current scenario and future directions. *American Journal of clinical Microbiology and Antimicrobials*, *2*(1), 1–2. Article 1030.

Goff, D. (2008). 65 years of ice-cream science. *International Dairy Journal*, *18*(7), 754–758.

Grover, S., Rashmi, H. M., Srivastava, A. K., & Batish, V. K. (2012). Probiotics for human health—New innovations and emerging trends. *Gut Pathogens*, *4*, 1–14.

Guarino, A., Guandalini, S., & Lo Vecchio, A. (2015). Probiotics for prevention and treatment of diarrhea. *Journal of Clinical Gastroenterology*, *49*(1), S37–S45.

Gupta, M., & Bajaj, B. K. (2017). Development of fermented oat flour beverage as a potential probiotic vehicle. *Food Bioscience*, *20*, 104–109.

Harper, A., Naghibi, M. M., & Garcha, D. (2018). The role of bacteria, probiotics and diet in irritable bowel syndrome. *Foods*, *7*, 13. doi: 10.3390/foods7020013.

Heidari, F., Abbaszadeh, S., & Mirak, S. E. M. (2017). Evaluation effect of combination probiotics and antibiotics in the prevention of recurrent Urinary Tract Infection (UTI) in women. *Biomedical and Pharmacology Journal*, *10*(2), 691–698. https://dx.doi.org/10.103005/bpj/1157.

Helland, M. H., Wicklund, T., & Narvhus, J. A. (2019). Growth and metabolism of selected strains of Probiotic bacteria, in maize porridge with added malted barley. *International Journal of Food Microbiology*, *91*, 147.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., et al. (2014). The international Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*, *11*, 506–514.

Hilliam, M. (2000). Functional food – How big is the market? *The World of Food Ingredients*, *12*, 50–52.

Holt, C., Carver, J. A., Ecroyd, H., & Thorn, D. C. (2013). Invited reviews: Caseins and the casein micelle: Their biological functions, structures and behaviour in foods. *Journal of Dairy Science*, *96*(10), 6127–6146. doi: 10.3168/jds. 2013-6831.

Hongu, N., Kim, A. S., Suzuki, A., Wilson, H., Tsui, K. U., & Park, S. (2017). Korean Kimchi: Promoting healthy meals through cultural tradition. *Journal of Ethnic Foods*, *4*(3), 172–180. http://classes.uleth.ca/200801/biol4500a/Thomas1.pdf.

Içier, F., Gunduz, G. T., Yılmaz, B., & Memeli, Z. (2015). Changes on some quality characteristics of fermented soy milk beverage with added apple juice. *LWT—Food Science and Technology*, *63*, 57–64.

Iebba, V., Nicoletti, M., & Schippa, S. (2012). Gut micro biota and the immune system: An intimate partnership in health and disease. *International Journal of Immunopathology and Pharmacology*, *25*, 823–833.

International Diabetes Federation. (2017). *IDF diabetes atlas*. Watermael-Boitsfort, Belgium: IDF.

Iqbal, M. Z., Qadir, M. I., Hussain, T., Janbaz, K. H., Khan, Y. H., & Ahmad, B. (2014). Probiotics and their beneficial effects against various diseases. *Pakistan Journal of Pharmaceutical Sciences*, *27*, 405–415.

Isolauri, E., Rautava, S., & Salminen, S. (2012). Probiotics in the development and treatment of allergic disease. *Gastroenterology Clinics of North America*, *41*, 747–762.

Jeanne, A., Lehter, V., Rhimi, M., Mangin, I., Peucelle, V., Abraham, A., et al. (2016). Beneficial metabolic effects of selected probiotics on diet-induced obesity and insulin resistance in mice are associated with improvement of dysbiotic gut micro biota. *Environmental Microbiology*, *18*(5), 1484–1497.

Ji, J., & Yang, H. (2020). Using probiotics as supplementation for *Helicobacter pylori* antibiotic therapy. *International Journal of Molecular Sciences*, *21*, 1136. doi: 10.3390/ijms21031136.

Kabeir, B. M., Yazid, A. M., Hakim, M. N., Khahatan, A., Shaborin, A., & Mustafa, S. (2009). Survival of *Bifidobacterium pseudocatenulatum* G4 during the storage of fermented peanut milk (PM) and skim milk (SM) products. *African Journal of Food Science*, *3*, 151–155.

Kandylis, P., Pissaridi, K., Bekatorou, A., Kanellaki, M., & Koutinas, A. A. (2016). Dairy and non-dairy probiotic beverages. *Current Opinion in Food Science*, *7*, 58–63.

Kaprasob, R., Kerdchoechuen, O., Laohakunjit, N., & Somboonpanyakul, P. (2018). B vitamins and Prebiotic fructo oligosaccharides of cashew apple fermented with probiotic strains *Lactobacillus* spp., *Leuconostoc mesenteroides* and *Bifidobacterium longum*. *Process Biochemistry*, *70*, 9–19.

Karimi, R., Mortazavian, A., & Cruz, A. (2011). Viability of probiotic microorganisms in cheese during production and storage: A review. *Dairy Science & Technology*, *91*, 283–308. doi: 10.1007/S13594-011-0005-x.

Katharina, E. S., Adolphi, B., Rochat, F., Barclay, D. V., De vrese, M., Açil, Y, et al. (2016). Effects of probiotics, prebiotics, and synbiotics on mineral metabolism in ovariectomized rats-impact of bacterial mass, intestinal absorptive area and reduction of bone turn-over. *NFS Journal*, *3*, 41–50.

Kedia, G., Wang, R., Patel, H., & Pandiella, S. S. (2007). Used of mixed cultures for the fermentation of cereal-based substrates with potential probiotic properties. *Process Biochemistry*, *42*, 65–70.

Kemsawasd, V., Chaikham, P., & Rattanasena, P. (2016). Survival of immobilized probiotics in chocolate during storage and with an *in vitro* gastrointestinal model. *Food Bioscience*, *16*, 37–43.

Kilic, S. (2008). *Lactic acid bacteria in dairy industry*. Bornova: Ege University Press. Agriculture Faculty Publication. p. 542.

Kilic, G. B., Agdas, K., Karahan, A. G., & Cakmakci, M. L. (2016). Effect of *Lactobacillus plantarum* AK4-11 and different grape varieties on the properties of hardaliye. *Tarim Bilimleri Dergisi*, *22*, 512–521. doi: 10.1501/Tarimbil_0000001409.

Klindt-toldam, S., Larsen, S. K., Saaby, L., Olsen, l. R., Svenstrup, G., Mullertz, A., et al. (2016). Survival of *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* HN019 encapsulated in chocolate during *in vitro* simulated passage of the upper gastrointestinal tract. *LWT*, *74*, 404–410.

Knudsen, B. K. E., Nerskov, N. P., Bolig, A. K., Hedemann, M. S., & Laerke, H. N. (2017). Dietary fibers and associated phytochemicals in cereals. *Molecular Nutrition & Food Research*, *61*, 1600518.

Kobyliak, N., Falalyreyeva, T., Boyko, N., Tsyryuk, O., Beregova, T., & Ostapchenko, L. (2018). Probiotics and nutraceuticals as a new frontier in obesity prevention and management. *Diabetes Research and Clinical Practice*, *141*, 190–199. https://doi.org/10.1016/j.diabres.2018.05.005.

Kumar, B. V., Vijayendra, S. V. N., & Reddy, O. V. S. (2015). Trends in dairy and non-dairy probiotic products – A review. *Journal of Food Science and Technology*, *52*, 6112–6124.

La Anh, N. (2015). Health promoting microbes in traditional Vietnamese fermented foods: A review. *Food Science and Human Wellness*, *4*(4), 147–167. doi: 10.1016/j.fshw.2015.08.004.

La Barz, M., Anhe, F. F., Varin, T. V., Desjardins, Y., Levy, E., Roy, D., et al. (2015). Probiotics as complementary treatment for metabolic disorders. *Diabetes & Metabolism Journal*, *39*, 291–303. http://dx.doi.org/10.4093/dmj.2015.39.4.291.

Lamba, J., Goomer, S., & Saxena, S. K. (2019). Study the lactic acid bacteria content in traditional fermented Indian drink: Kanji. *International Journal of Gastronomy and Food Science*, *16*, 100143. doi: 10.1016/jijgfs.2019.100143.

Larsen, N., Vogensen, F. K., Van-den-Berg, F. W., Nielsen, D. S., Andresen, A. S., Pedersen, B. K., et al. (2010). Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One*, *5*, 1–10.

Lebensmittelsicherheits – und Verbraucherschutzgesetz – Bundesrecht Konsolidiert. Rechsinformationssystem des Bundes. 2019, p47.

Lebos-Pavunc, A., Penava, L., Ranilovic, J., Novak, J., Banic, M., Butorac, K., et al. (2019). Influence of dehydrated wheat/rice cereal matrices on Probiotic activity of *Bifidobacterium animalis* spp. Lactis BB-12. *Food Technology and Biotechnology*, *57*, 147.

Li, P., & Gu, Q. (2016). Complete genome sequence of *Lactobacillus plantarum* ZJ95, a potential probiotic strain producing bacteriocins and B-group vitamin riboflavin. *Journal Biotechnology*, *229*, 1–2.

Li, Z., Teng, J., Lyu, Y., Hu, X., Zhao, Y., & Wang, M. (2019). Enhanced antioxidant activity for apple juice fermented with *Lactobacillus plantarum* ATCC14917. *Molecules*, *24*, 51.

Lieske, J. C. (2017). Probiotics for prevention of urinary stones. *Annals of Translational Medicine*, *5*(2), 29. doi: 10.21037/atm.2016.11.86.

Liu, X., Kuda, T., Takahashi, H., & Kimura, B. (2018a). Bacterial and fungal micro biota of spontaneously fermented Chinese products. Rubbing milk cake and Yan-cai vegetable pickles. *Food microbiology*, *72*, 106–111.

Liu, Y., Chen, H., Chen, W., Zhong, Q., Zhang, G., & Chen, W. (2018b). Beneficial effects of tomato juice fermented by *Lactobacillus plantarum* and *Lactobacillus casei:* antioxidation, antimicrobial effect, and volatile profiles. *Molecules*, *23*, 2366.

Majumdar, R. K., Roy, D., Bejjanki, S. K., & Bhaskar, N. (2016). An overview of some ethnic fermented fish products of eastern Himalayan region on India. *Journal of Ethnic Foods*, *3*(4), doi: 10.1016/j.jef.2016.12.003.

Mantzourani, I., Kazakos, S., Terpou, A., Alexopoulos, A., Bezirtzoglou, E., Bekatorou, A., et al. (2019). Potential of the probiotic *Lactobacillus plantarum* ATCC14917 strain to produce functional fermented pomegranate juice. *Foods*, *8*, 4.

Maria, K., Dimitrios, B., Stavroula, K., et al. (2013). Health benefits of probiotics: A review. *ISRN Nutrition*, *2*, 185–188. doi: 10.5402/2013/481651.

Martinez, R. C., Franceschini, S. A., Patta, M. C., Quintana, S. M., Candido, R. C., Ferreira, J. C., et al. (2009). Improved treatment of vulvovaginal candidiasis with fluconazole plus probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14. *Applied Microbiology Letters*, *48*, 269–274.

Menezes, A. G. T., Ramos, C. L., Dias, D. R., & Schwan, R. F. (2018). Combination of probiotic yeast and lactic acid bacteria as starter culture to produce maize-based beverages. *Food Research International*, *111*, 187–197.

Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., et al. (2011). Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longam* R0175) in rats and human subjects. *British Journal of Nutrition*, *105*, 755–765.

Meybodi, N. M., & Mrtazavian, A. M. (2017). Probiotic supplements and food products: A comparative approach. *Biochemical Pharmacology*, *6*, 2. doi: 10.4172/2167-050.1000227.

Mirkovic, M., Seratlc, S., Kilcawley, K., Mannion, D., Mirkovic, N., & Radulovic, Z. (2018). The sensory quality and volatile profile of dark chocolate enriched with encapsulated probiotic *Lactobacillus plantarum* bacteria. *Sensors*, *18*, 2570.

Mojgani, N., Fatimah, H. F., & Vaseji, N. (2015). Characterization of indigenous *Lactobacillus* strains for probiotic properties. *Jundishapur Journal of Microbiology*, *8*, 1–2.

Mustafa, S., Shaborin, A., Kabeir, B. M., Yazid, A. M., Hakim, M. N., & Khahtanan, A. (2009). Survival of *Bifidobacterium pseudocatenulatum* G4 during the storage of fermented peanut milk (PM) and skim milk (SM) products. *African Journal of Food Science*, *3*, 150–155.

Nahidul-Islam, S. M., Kuda, T., Takahashi, H., & Kimura, B. (2018). Bacterial and fungal micro biota in traditional Bangladeshi fermented milk products analysed by culture-dependent and culture-independent methods. *Food Research International*, *111*, 431–437. doi: 10.1016/j.foodres.2018.05.048.

Nazhand, A., Souto, E. B., Lucarini, M., Souto, S. B., Durazzo, A., & Santini, A. (2020). Ready to use therapeutical beverges: Focus on functional beverages containing probiotics, prebiotics and synbiotics. *Beverages*, *6*, 26. doi: 10.3390/beverages6020026.

Nazzaro, F., Fratinni, F., Sada, A., & Orlando, P. (2008). Synbiotic potential of carrot juice supplemented with *Lactobacillus* spp. and inulin or fructooligosaccharides. *Journal of the Science of Food and Agriculture*, *88*, 2271–2276.

Nebesny, E., Żyżelewicz, D., Motyl, I., & Libudzisz, Z. (2007). Dark chocolates supplemented with *Lactobacillus* strains. *European Food Research and Technology*, *225*, 33–42.

Nguyen, B. T., Bujna, E., Fekete, N., Tran, A. T., Rezessy-Szabo, J. M., Prasad, R., & Nguyen, Q. D. (2019). Probiotic beverage from pineapple juice fermented with *Lactobacillus* and *Bifidobacterium* strains. *Frontiers in Nutrition*, *6*, 54.

Nogueiras, R., Romero-pica, A., Vazquez, M. J., Novelle, M. G., & Lopez, M. (2012). The opioid system and food intake: Homeostatic and hedonic mechanisms. *Obesity Facts*, *5*, 196–207.

Nualkaekul, S., Deepika, G., & Charalampopoulos, D. (2012). Survival of freeze dried *Lactobacillus plantarum* in instant fruit powders and reconstituted fruit juices. *Food Research International*, *48*, 627–633.

Oh, Y. J., & Jung, D. S. (2015). Evaluation of probiotic properties of *Lactobacillus* and *Pediococcus* strains isolated from Omegisool, a traditionally fermented millet alcoholic beverage in Korea. *LWT—Food Science and Technology*, *63*, 437–444.

Ong, L., & Shah, N. P. (2009). Probiotic cheddar cheese: Influence of ripening temperatures on survival of probiotic microorganisms. Cheese composition and organic acid profiles. *LWT—Food Science and Technology*, *42*, 1260–1268.

Ortiz, Y., Garcia-Amezquita, E., Acosta, C. H., & Sepulveda, D. R. (2017). Functional dairy products. In G. Barbosa-canovas (Ed.), *Global food security and wellness* (pp. 67–103). New York: Springer.

Ouwehand, C., & Tennila, J. (2016). Probiotics and antibiotic use. In R. R. Watson, & V. R. Preedy (Eds.), *Probiotics, probiotics and synbiotics: Bioactive foods in health promotion* (pp. 271–277). London: Elsevier Inc.

Ozden, A. (2008). Other fermented dairy products: Bio-yogurt – probiotic yogurt. *Guncel Gastroenteroloji*, *12*(3), 169–181.

Ozer, B. H., & Kirmaci, H. A. (2010). Functional milks and dairy beverages. *International Journal of Dairy Technology*, *63*, 1–15.

Ozyurt, V. H., & Otles, S. (2014). Properties of probiotics and encapsulated probiotics in food. *Acta Scientiarum Polonorum Technologia Alimentaria*, *13*(4), 413–424.

Pace, F., Pace, M., & Quartarone, G. (2015). Probiotics in digestive diseases: Focus on *Lactobacillus* GG. *Minerva Gastroenterologica e Dietologica*, *61*, 1–22.

Pandey, K. R., Naik, S. R., & Vakil, B. V. (2015). Probiotics, prebiotics and synbiotics—A review. *Journal of Food Science and Technology*, *52*, 7577–7587.

Panghal, A., Janghu, S., Virkar, K., Gat, Y., Kumar, V., & Chhikara, N. (2018). Potential non-dairy probiotic products—A healthy approach. *Food Bioscience*, *21*, 80–89.

Papanna, S., & Agarwal, R. (2011). A potent probiotic strain from cheddar cheese. *Indian Journal of Microbiology*, *51*(3), 251–258. doi: 10.1007/s12088-011-0072-y.

Park, S., & Bae, J. -H. (2015). Probiotics for weight loss: A systematic review and meta-analysis. *Nutrition Research*, *35*(7), 566–575.

Parvaneh, K., Jamaluddin, R., Karimi, G., & Erfani, R. (2014). Effect of probiotics supplementation on bone mineral content and bone mass density. *Scientific World Journal*, *22*, 595962.

Pavli, F. G., Argyri, A. A., Chorianopoulos, N. G., Nychas, G. J. E., & Tassou, C. C. (2019). Evaluation of *Lactobacillus plantarum* L125 strain with probiotic potential on physicochemical, microbiological and sensorial characteristics of dry-fermented sausages. *LWT*, *118*, 108810.

Pereira, A. L. F., & Rodrigues, S. (2018). Turning fruit juice in to probiotic beverages. *FruitJuices*. Amsterdam, The Netherland: Elsevier. pp. 279–287.

Pereira, A. L. F., Maciel, T. C., & Rodrigues, S. (2011). Probiotic beverage from cashew apple juice fermented with *Lactobacillus casei*. *Food Research International*, *44*, 1276–1283.

Peres, C. M., Hernandez Mendonza, A., Bronze, M. R., Peres, C., & Xavier Malcata, F. (2015). Synergy of olive bioactive phytochemicals and probiotic strain in control of *Escherichia coli*. *LWT—Food Science and Technology*, *64*, 938–945.

Pontonia, E., & Rizzello, C. G. (2019). Minor and ancient cereals: Exploitation of the nutritional potential through the use of selected starters and sourdough fermentation. In V. Preedy, R. Watson, & V. Patel (Eds.), *Flour and Breads and Their Fortification in Health and Disease Prevention* (pp. 443–452). Amsterdam, The NetherlandsLondon, UK: Elsevier IncAcademic Press.

Pool-Zobel, B. L. 1., Neudecker, C., Domizlaff, I., Ji, S., Schillinger, U., et al. (1996). *Lactobacillus* and *Bifidobacterium* mediated antigenotoxicity in the colon of rats. *Nutrition and Cancer*, *36*(7), 365–380.

Prado, F. C., Parada, J. L., Pandey, A., & Soccol, C. R. (2008). Trends in dairy and non-dairy probiotic beverages. *Food Research International*, *41*, 111–123.

Quigley, L., O'Sullivan, O., Beresford, T. P., Ross, R. P., Fitzgerald, G. F., & Cotter, P. D. (2011). Molecular approaches to analysing the microbial composition of raw milk and raw milk cheese. *International Journal of Food Microbiology*, *150*, 81–94. doi: 10.1016/j.ijfoodmicro.2011.08.001.

Raghuwanshi, S., Misra, S., Sharma, R., & Bisen, P. S. (2015). Indian perspective for probiotics: A review. *Indian Journal of Dairy Science*, *68*, 195–205.

Rajendran, C. S. C., Chamlagain, B., Kariluoto, S., Piironen, S., & Saris, P. E. J. (2017). Bio fortification of riboflavin and folate in idli batter, based on fermented cereal and pulse, by *Lactococcus lactis* N8 and *Saccharomyces boulardii* SAA655. *Applied Microbiology*, *122*, 1663–1671.

Rakin, M., Vukasinovic, M., Siler-Marinkovic, S., & Maksimovic, M. (2007). Contribution of lactic acid fermentation to improved nutritive quality vegetable juices enriched with brewer's yeast autolysate. *Food Chemistry*, *100*, 599–602.

Ramos, C. L., Thorsen, L., Schwan, R. F., & Jespersen, L. (2013). Strain-specific probiotics properties of *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus brevis* isolates from Brazilian food products. *Food Microbiology*, *36*(1), 22–29. https://dx.doi.org/10.1016/j.fm.2013.03.010.

Redondo-Useros, N., Gheorghe, A., Diaz-Prieto, L. E., Villavisencio, B., Marcos, A., & Nova, E. (2019). Associations of probiotic fermented milk (PFM) and yogurt consumption with *Bifidobacterium* and *Lactobacillus* components of the gut micro biota in healthy adults. *Nutrients*, *11*, 651. doi: 10.3390/nul1030651.

Reid, G., & Devillard, E. (2004). Probiotics for mother and child. *Journal of Clinical Gastroenterology*, *38*, S94–S101.

Renuka, B., Kulkarni, S. G., Vijayanand, P., & Prapulla, S. G. (2009). Fructo oligosaccharide fortification of selected fruit juice beverages: Effect on the quality characteristics. *LWT—Food Science and Technology*, *43*, 1031–1033.

Rivera-Espinoza, Y., & Gallardo-Navarro, Y. (2010). Non-dairy probiotic products. *Food Microbiology*, *27*, 1–11.

Riviere, A., Selak, M., Lantin, D., Leroy, F., & De Vuyst, L. (2016). *Bifidobacteria* and butyrate producing colon bacteria: Importance and strategies for their stimulation in the human gut. *Frontiers in Microbiology*, *7*, 979.

Rodgers, S. (2008). Novel applications of live bacteria in food services: Probiotics and protective cultures. *Trends in Food Science and Technology*, *19*, 188–197.

Rosa, D. D., Dias, M. M., Grzeskowiak, L. M., Reis, S. A., Conceicao, L. L., & Peluzio, M. D. C. G. (2017). Milk Kefir: Nutritional, microbiological and health benefits. *Nutrition Research Reviews*, *30*(1), 82–96.

Rosado, J. L. (1996). Yogurt as a source of lactose auto digestion. *Revista de Investigación Clínica*, *48*, 636.

Ruiz, J. N., Villanueva, N. D. M., Favaro-Trindade, C. S., & Contreras-Castillo, C. J. (2014). Physicochemical, microbiological and sensory assessments of Italian salami sausages with probiotic potential. *Scientia Agricola*, *71*, 204–211.

Saarela, M., Virkajarvi, I., Nohynek, I., Vaari, A., & Matto, J. (2006a). Fibres as carriers for *Lactobacillus rhamnosus* during freeze-drying and storage in apple juice and chocolate-coated breakfast cereals. *International Journal of Food Microbiology*, *112*, 171–178.

Saarela, M., Virkarjarvi, I., Alakomi, H. L., Matilla, P. S., & Matto, J. (2006b). Stability and functionality of freeze-dried probiotic *Bifidobacterium* cells during storage in juice and milk. *International Dairy Journal*, *16*, 1477–1482.

Sameshima, T., Magome, C., Takeshita, K., Arihara, K., Itoh, M., & Kondo, Y. (1998). Effect of intestinal *Lactobacillus* starter cultures on the behaviour of *Staphylococcus aureus* in fermented sausage. *International Journal of Food Microbiology*, *41*, 1–7.

Sarkar, P., Lohithkumar, D. H., Dhumal, C., Panigrahi, S. S., & Choudhary, R. (2015). Traditional and ayurvedic foods of Indian origin. *Journal of Ethnic Foods*, *2*, 97–109. http://dx.doi.org/10.1016/j.jef.2015.08.003.

Saubade, F., Hemery, Y. M., Guyot, J. P., & Humblot, C. (2017). Lactic acid fermentation as a tool for increasing the folate content of foods. *Critical Reviews in Food Science and Nutrition*, *57*, 3894–3910.

Schrezenmeir, J., & de Vrese, M. (2001). Probiotics, prebiotics and synbiotics – Approaching a definition. *The American Journal of Clinical Nutrition*, *73*, 361s–364s.

Semjonovs, P., Denina, I., Fomina, A., Sakirova, L., Auzina, L., Patetko, A., et al. (2013). Evaluation of *Lactobacillus reuteri* strains for pumpkin (*Cucurbita pepo* L.) juice fermentation. *Biotechnology*, *12*, 202–208.

Sendra, E., Fayos, P., Lario, Y., Femandez-Lopez, J., Sayas-Barbera, E., & Perez-Alvarez, J. A. (2008). Incorporation of citrus fibers in fermented milk containing probiotic bacteria. *Food Microbiology*, *25*, 13–21.

Sharma, S., Arora, M., & Baldi, A. (2013). Probiotics in India: Current status and future prospects. *Pharmaspire*, *4*, 68–77.

Sharma, M., Mridula, D., & Gupta, R. (2014). Development of sprouted wheat based probiotic beverage. *Journal of Food Science and Technology*, *51*, 3926–3933.

Sheehan, V. M., Ross, P., & Fitzgerald, G. F. (2004). Assessing the acid tolerance and the technological robustness of probiotic cultures for fortification in fruit juices. *Innovative Food Science and Emerging Technologies*, *8*(2), 279–284.

Sheela, T., & Suganya, R. (2012). Studies on anti-diarrhoeal activity of synbiotic plums juice. *International Journal of Scientific and Research Publications*, *2*, 1–5.

Sherahi, M. H. A., Shahidi, F., Tabatabaee, F., & Hashemi, S. M. B. (2018). Effect of *Lactobacillus plantarum* on olive and olive oil quality during fermentation process. *LWT—Food Science and Technology*, *89*doi: 10.1016/j.jwt.2017.10.025.

Shiby, V. K., & Misra, H. N. (2013). Fermented milks and milk products as functional foods: A review. *Critical Reviews in Food Science and Nutrition*, *53*(5), 482–496.

Shori, A. B. (2016). Influence of food matrix on the viability of probiotic bacteria: A review based on dairy and non-dairy beverages. *Food Bioscience*, *13*, 1–8.

Shukla, P., & Kushwaha, A. (2017). Development of probiotic beverage from whey and orange juice. *Journal of Nutrition & Food Sciences*, *7*(5), 1000629. doi: 10.4172/2155-9600.1000629.

Side, C. (2006). Overview on marketing of functional food in Europe. In *Functional food network general meeting*.

Sidira, M., Mitropoulou, G., Galanis, A., Kanellaki, M., & Kourkoutas, Y. (2019). Effect of Sugar Content on Quality Characteristics and Shelf-Life of Probiotic Dry-Fermented Sausages Produced by Free or Immobilized *Lactobacillus casei* ATCC 393. *Foods*, *8*, 219.

Slavin, J. L., & Lioyd, B. (2012). Health benefits of fruits and vegetables. *Advances in Nutrition*, *3*, 506–516.

Slima, S. B., Ktari, N., Triki, M., Trabelsi, I., Abdeslam, A., Moussa, H., et al. (2018). Effects of probiotic strains, *Lactobacillus plantarum* TN8 and *Pediococcus acidilactici* on microbiological and physico-chemical characteristics of beef sausages. *LWT*, *92*, 195–203.

Sridevi, J., Halami, P. M., & Vijayendra, S. V. N. (2010). Selection of starter cultures for Idli batter fermentation and their effect on quality of idli. *Journal of Food Science and Technology*, *47*, 557–563. doi: 10.1007/s13197-1010-0101-6.

Stephenie, W., Kebeir, B., Shuhaimi, M., Rosfarizan, M., & Yazid, A. (2007). Growth optimization of a probiotic candidate, *Bifidobacterium pseudocatenulatum* G4 in milk medium using response surface methodology. *Biotechnology and Bioprocess Engineering*, *12*, 106–113.

Sullivan, A., & Nord, C. E. (2005). Probiotics and gastrointestinal diseases. *Journal of Internal Medicine*, *257*, 78–92.

Swain, M. R., Anandharaj, M., Ray, R. C., & Rani, R. P. (2014). Fermented fruit and vegetables of Asia: A potential source of probiotics. *Biotechnology Research International*, *2014*, 1–19. doi: 10.1155/2014/250424.

Swieca, M., Kordowska-Wiater, M., Pytka, M., Gawlik-Dziki, U., Bochnak, J., Złotek, U., et al. (2018). *Lactobacillus plantarum* 299V improves the microbiological quality of legume sprouts and effectively survives in these carriers during cold storage and in vitro digestion. *PLoS One*, *13*, e0207793.

Szajewska, H. (2016). What are the indications for using probiotics in children? *Archives of Disease in Childhood*, *101*, 398–403.

Tamang, J. P., Shin, D. H., Jung, S. -J., & Chae, S. -W. (2016). Functional properties of microorganisms in fermented food. *Frontiers in Microbiology*, *7*. https://dx.doi.org/10.3389/fmicb.2016.00578.

Tillisch, K. (2014). The effects of gut micro biota on CNS function in humans. *Gut Microbes*, *5*, 404–410.

Toma, M. M., & Pokrotnieks, J. (2006). Probiotics as functional food: Microbiological and medical aspects. *Acta Universitatis Latviensis*, *710*, 117–129.

Torres-Fuentes, C., Schellekens, H., Dinan, T. G., & Cryan, J. F. (2015). A natural solution for obesity bio actives for the prevention and treatment of weight gain—A review. *Nutritional Neurosciences*, *18*(2), 49–65.

Tripathi, M. K., & Giri, S. K. (2014). Probiotic functional foods: Survival of probiotics during processing and storage. *Journal of Functional Foods*, *9*, 225–241.

Tsafrakidou, P., Michaelidou, A. M., & Biliaderis, C. G. (2020). Fermented cereal based products: Nutritional aspects, possible impact on gut microbiota and health implications. *Foods*, *9*, 734. doi: 10.3390/foods9060734.

Tsen, J. H., Lin, Y. P., & King, A. E. (2009). Response surface methodology optimisation of immobilized *Lactobacillus acidophilus* banana puree fermentation. *International Journal of Food Science & Technology*, *44*, 120–127.

Umbrello, G., & Esposito, S. (2016). Micro biota and neurologic diseases: Potential effects of probiotics. *Journal of Translational Medicine*, *14*, 1–11.

Ustun, C. (2009). An ancient Turkish beverage: Kimiz (Koumiss). *Türklük Bilimi Araştırmaları*, *26*, 247–255.

Vaikunthanathan, T., Safinia, N., Lombardi, G., & Lechler, R. I. (2016). Microbiota, immunity and the liver. *Immunology Letters*, *171*, 36–49.

Valero-Cases, E., Roy, N. C., Frutos, M. J., & Anderson, R. C. (2017). Influence of the fruit juice carriers on the ability of *Lactobacillus plantarum* DSM20205 to improve in vitro intestinal barrier integrity and its probiotic properties. *Journal of Agricultural and Food Chemistry*, *65*, 5632–5638.

Vecchione, A., Celandroni, F., Mazzantini, D., Senesi, S., Lupetti, A., & Ghelardi, E. (2018). Compositional quality and potential gastrointestinal behaviour of probiotic products commercialized in Italy. *Frontiers of Medicine*, *5*, 59.

Wang, L., Guo, M. J., Gao, Q., Yang, J. F., Yang, L., Pang, X. L., et al. (2018). The effects of probiotics on total cholesterol—A meta-analysis of randomized controlled trials. *Medicine*, *97*(5), e9679. http://dx.doi.org/10.1097/MD.0000000000009679.

Wilson, E., Seo, C., Ahahbazi, A., & Ibrahim, S. (2004). Survival and growth of probiotic cultures in sour cream products. In *IFT annual meeting* (pp. 17A). .

Wu, Y., Zhu, C., Chen, Z., Chen, Z., Zhang, W., Ma, X., et al. (2016). Protective effects of *Lactobacillus plantarum* on epithelial barrier disruption caused by enterotoxigenic *Escherichia coli* in intestinal porcine epithelial cells. *Veterinary Immunology and Immunopathology*, *172*, 55–63.

Wuyts, S., Van Beeck, W., Allonsius, C. N., van den Broek, M. F, & Lebeer, S. (2020). Applications of plant based fermented foods and their microbes. *Current Opinion in Biotechnology*, *61*, 45–52.

Xu, X., Bao, Y., Wu, B., Lao, F., Hu, X., & Wu, J. (2019). Chemical analysis and flavour properties of blended orange, carrot, apple and Chinese jujube juice fermented by selenium-enriched probiotics. *Food Chemistry*, *289*, 250–258.

Yahfoufi, N., Mallet, J. F., Graham, E., & Matar, C. (2018). Role of probiotics and prebiotics in immunomodulation. *Current Opinion in Food Science*, *20*, 82–91. http://doi.org/10.1016/j.cofs.2018.04.006.

Yang, X., Zhou, J., Fan, L., Qin, Z., Chen, Q., & Zhao, L. (2018). Antioxidant properties of a vegetable–fruit beverage fermented with two *Lactobacillus plantarum* strains. *Food Science and Biotechnology*, *27*, 1719–1726.

Yoo, J. Y., & Kim, S. S. (2016). Probiotics: Present status and future perspectives on metabolic disorders. *Nutrients*, *8*(173), 1–20.

Yoon, K. Y., Woodams, E. E., & Hang, Y. D. (2004). Probiotication of tomato juice by lactic acid bacteria. *Journal of Microbiology (Seoul, Korea)*, *42*, 315–318.

Yoon, K. Y., Woodams, E. E., & Hang, Y. D. (2005). Fermentation of beet juice by beneficial lactic acid bacteria. *LWT—Food Science and Technology*, *38*, 73–75.

Yoon, K. Y., Woodamns, E. E., & Hang, Y. D. (2006a). Production of probiotic cabbage juice by lactic acid bacteria. *Bioresource Technology*, *9*, 1427–1430.

Yoon, K. Y., Woodams, E. E., & Hang, Y. D. (2006b). Production of probiotic cabbage juice by lactic acid bacteria. *Bioresource Technology*, *97*, 1427–1430.

Zarić, D. B., Bulatović, M. L., Rakin, M. B., Krunić, T. Z., Lonćarevi, I. S., & Pajin, B. S. (2016). Functional, rheological and sensory properties of probiotic milk chocolate produced in a ball mill. *RSC Advances*, *6*, 13934–13941.

Zhao, W., Liu, Y., Latta, M., Ma, W., Wu, Z., & Chen, P. (2019). Probiotics database: A potential source of fermented foods. *International Journal of Food Properties*, *22*(1), 197–216. doi: 10.1080/10942912.2019.1579737.

Ziyadi, S., Bastani, P., Homayouni, A., Charandabi, S. M., & Mallah, F. (2016). Probiotics and usage in urinary tract infection. In R. R. Watson, & V. R. Preedy (Eds.), *Probiotics, probiotics and synbiotics: Bioactive foods in health promotion* (pp. 827–830). London: Elsevier Inc.

Part IV

Probiotics in Health and Diseases

12. Probiotic Microorganism: A Promising and Innovative Tool for Cancer Prevention and Therapy 187
13. Psychobiotics: A Newer Approach Toward the Treatment of Neurodevelopmental Disorders 203
14. Probiotics, Diet, and Gut Microbiome Modulation in Metabolic Syndromes Prevention 217
15. *Bacillus* Species—Elucidating the Dilemma on Their Probiotic and Pathogenic Traits 233
16. Probiotic Fortified Seaweed Silage as Feed Supplement in Marine Hatcheries 247
17. Secondary Metabolites From Probiotic Metabolism 259
18. Bacteriocins Produced by Probiotic Microorganisms 277
19. Probioactives: Bacteriocin and Exopolysaccharides 293
20. Probiotics in Shrimp Aquaculture 309
21. Prospective Approaches of *Pseudonocardia alaniniphila* Hydrobionts for *Litopenaeus vannamei* 327
22. Probiotics as a Growth Promotant for Livestock and Poultry Production 349
23. Small- and Large-Scale Production of Probiotic Foods, Probiotic Potential and Nutritional Benefits 365
24. Lactic Acid Bacteria in Fermented Food 397
25. Commercially Available Probiotics and Prebiotics Used in Human and Animal Nutrition 417
26. New Formulations and Products in Prebiotic Food 437
27. Therapeutic Potential of Different Probiotic Foods 449
28. Main Technological Challenges Associated With the Incorporation of Probiotic Cultures into Foods 479
29. Effective Probiotic Delivery: Current Trends and Future Perspectives 497
30. Industrial Requirements and Other Techno-functional Traits of Probiotics 519

Chapter 12

Probiotic Microorganism: A Promising and Innovative Tool for Cancer Prevention and Therapy

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1 Introduction

Cancer is a disease where cells divide and grow uncontrollably destroying the body tissues by manipulating the proliferation as well as cell death mechanisms. Cancer is the second most common reason for worldwide mortality after the cardiovascular disorder and accounts for 23% and 7% deaths in the United States and India, respectively (Jemal et al., 2008). Gastrointestinal (GI) cancer accounts for 25% of all cancers and for 9% of all causes of cancer death in worldwide (Rawla & Barsouk, 2019; Mego, Holec, & Drgona, 2013). GI cancer is characterized by malignant conditions of the GI tract and other organs, including cancers of the esophagus (eighth most common), gallbladder, liver, pancreas, gastric (fourth most common), small intestine, colon, and rectum (third most common) (Pourhoseingholi, Vahedi, & Baghestani, 2015). Chemotherapeutic drugs are the standard treatment available at present. Systemic use of these chemotherapeutic options is associated with various adverse effects. The unwanted side effects coupled with the nonspecific nature of small molecule inhibitors are the major obstacles preventing optimal use of these therapies in clinics. Alternate preventive and therapeutic agents of microbial origin that can circumvent or minimize the adverse events in cancer treatment are being purported as prophylaxis and therapies (Bazuro, Torino, & Gasparini, 2008; Mego, Holec, & Drgona, 2013).

Probiotics are live microorganisms, which when administered in adequate amounts, confer a health benefit to the host. Although probiotics were important components and have been used in human diet for centuries, at present there is increase in basic and clinical research, and they are being incorporated into an ever-expanding array of foods, nutritional supplements, and pharmaceutical products globally. The concept of probiotics has gained scientific interest of health-care communities that have started giving serious attention to its beneficial health effects. Recent research suggests that probiotics induce health benefits to the host mainly by maintaining the microbial balance of the intestine, reducing pathogenic GI microorganisms, and also modulating immune system by managing antiinflammatory responses (Fig. 12.1) of human body in response to pathogenic or genetic changes. Probiotic lactobacilli of human origin, including *Lactobacillus acidophilus*, *Lactobacillus rhamnosus GG* (LGG), *Lactobacillus salivarius*, *Bifidobacterium bifidum*, and many others beneficial bacteria, have been shown antibacterial activity against *Helicobacter pylori* and other pathogenic infection in GI tract (Richman et al., 2017; Zhu & Liu, 2017). In addition, metabolites released from probiotic microorganism show pharmacological activities that can be utilized in the treatment of various human diseases. The symbiotic relationship between gut-microbiota and host is prerequisite for proper nutrient digestion, their absorption, and maintenance of normal metabolic profile (Ritchie & Romanuk, 2012; Blaser, 2014). Dysbiosis of the microbial community has been linked with various diseased states, including carcinogenesis, chemotherapeutic metabolism, and cancer-related symptoms (Goulet, 2015; Garrett, 2015). Onset of the gastric cancer (GC) is dependent on various factors from low socioeconomic status to *H. pylori* infection. *H. pylori* is

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00012-5
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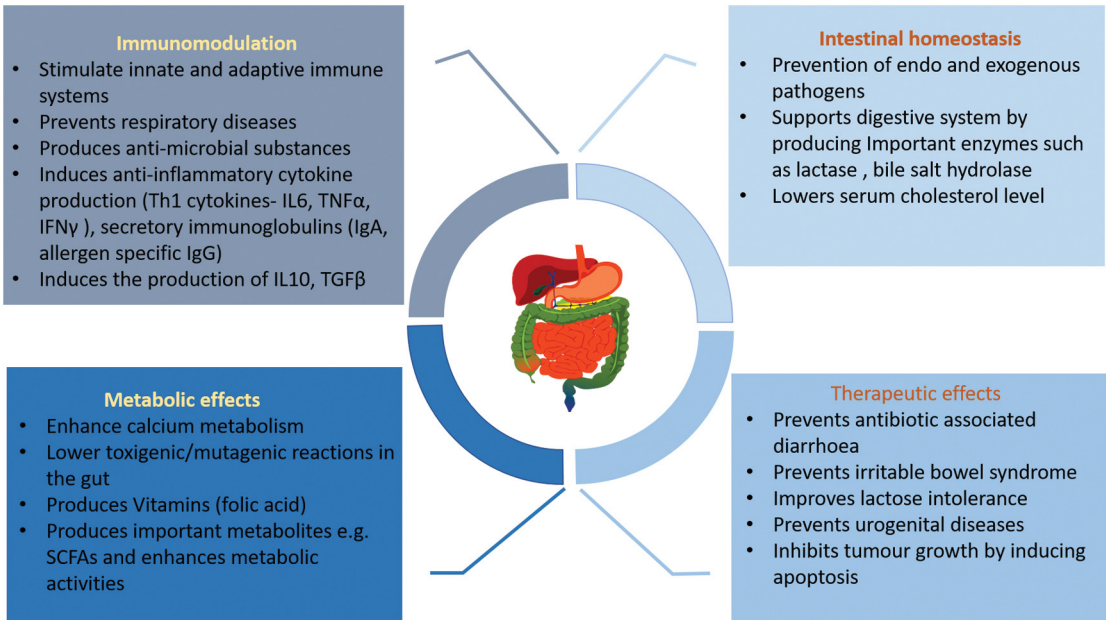


FIGURE 12.1 Health Benefits associated with Probiotics.

considered as type I carcinogenic agent and *H. pylori*–induced GC is characterized by damaged mucosa and facilitates the formation of mucosa-associated lymphoid tissue-lymphoma gastric tumors (Kelly, Lyon, Yoon, & Horgas, 2016). Reconstruction of damaged intestinal barrier and production of mucin are important attributes of probiotic organisms.

The interaction within gut microbiota leads to several anticancer effects such as the suppression of microbial population that produces mutagens/carcinogens, neutralization of carcinogens by direct binding and antiproliferative effects via regulation of apoptosis and cell differentiation (Choi, Kim, & Han, 2006; Kelly, Lyon, & Yoon, 2016; Kim, Kim, & Lee, 2007; Liu & Pan, 2010; Nami, Abdullah, & Haghshenas, 2014; Sadeghi-Aliabadi, Mohammadi, & Fazeli, 2014; Tiptiri-Kourpeti, Spyridopoulou, & Santarmaki, 2016). Considering the overwhelming accumulation of evidence pertaining to beneficial health effects of probiotics, it is evident that probiotic intervention could become a new adjuvant or even therapeutic approach to prevent and treat various cancers. Although direct effects of probiotics on cancer prevention and treatment is yet to be established, in this chapter an effort has been made to elucidate the various possibilities of probiotics in cancer prevention and as potential therapeutics.

2 Chronic inflammation is a major oncogenic stimulant

There are several factors that are responsible for cancer development such as external factors (90%–95%) and genetic defects (5%–10%) (alterations in the normal cell processes like cell cycle, cell signaling, and differentiation) making it a multifactorial disease (Jemal et al., 2008). Chronic inflammation is one of the major external factors that contributes in the development of various types of cancers. The functional relationship between inflammation and cancer is not new, it dates back to 1863 when Virchow (1989) hypothesized that the origin of cancer was at sites of chronic inflammation. Inflammation plays important roles in both physiological and pathological processes such as wound healing and infection. A fine balance exists between pro- and antiinflammatory cytokines that makes the inflammation associated with wound healing self-limiting in itself. However, any kind of dysregulation of limiting factors can disturb the balance and results in abnormalities and ultimately neoplastic growth.

Leukocytes and sometimes eosinophils are the first recruited effectors that are transported to any wound or infection site through venous system (Kita, 2011). After leukocytes have been recruited, macrophages that have differentiated from monocytes are guided by the chemotactic factors toward the site of tissue injury. These macrophages later become the major stimulator for the secretion growth factors and cytokines. Inflammatory mediators, such as histamine, cytokines, and proteases, released from mast cells, lead to the acute inflammatory responses. These inflammatory cell components, such as diverse leukocyte population—for example, neutrophils, dendritic cells (DCs), macrophages, eosinophils, and mast cells, as well as lymphocytes capable of producing array of cytokines, cytotoxic mediators, including reactive oxygen species (ROS), serine and cysteine proteases, tumor necrosis factor-α (TNF-α), interleukins (ILs) and interferons (IFNs), are also be found in tumors and their surrounding microenvironment (Homey, Müller, & Zlotnik, 2002). Tumor-associated

macrophages derived from monocytes are also responsible for secreting some important factors such as potent angiogenic and lymphangiogenic growth factors, cytokines, and proteases that facilitate neoplastic progression (Schoppmann et al., 2002).

Apart from influencing tumor microenvironment (TME) itself, cancer progression has been shown to be modulated by inflammatory responses by either inhibiting or stimulating the oncogenic signaling. Chronic inflammation induced by responses such as oxidative stress, endoplasmic reticulum (ER) stress, and mitochondrial stress play major role in the development and progression of cancer (Lin, Jiang, Chen, Zhao, & Wei, 2019; Boland, Chourasia, & Macleod, 2013). An imbalance between free radicals and reactive metabolites, the so-called oxidants or ROS, and their elimination induce the development of oxidative stress and further accelerate the chances of onset of cancer. ROS induces cellular proliferation, cellular migration, DNA damage, leading to genetic lesions that initiate tumorigenicity and subsequent tumor progression. Multiple cellular signaling pathways such as MAPK/AP-1 and NF-κB pathways have also been significantly influenced by various oxidants (Müller, Cahill, Rupec, Baeuerle, & Nordheim, 1997). ROS-induced oxidative stress influences the development of chronic inflammatory conditions in several ways. Induction of cyclooxygenase-2 and inducible nitric oxide synthase, aberrant expression of inflammatory cytokines (TNF, IL-1, IL-6), and chemokines [IL-8, CXC chemokine receptor 4 are the major ways through which oxidative stress induces inflammation (Hussain & Harris, 2007)]. Oxidative stress also augments metastasis by activating NF-κB, which, in turn, controls the expression of a cell surface protein in endothelial and epithelial cells called intercellular adhesion protein-1 (ICAM1) (Roebuck, 1999).

ER stress is also involved in inducing inflammatory conditions and ultimately development and progression of different cancer types. Chronic inflammation induces chronic ER stress and results in endogenous or exogenous damage to cells and activates unfolded protein response (UPR). The UPR reaction stimulates tumor cells to secrete metalloproteinases that bind to specific cytokines and angiogenic factors. UPR response can also promote the production of ROS in the ER and induce inflammatory responses through three branches of UPR in ER stress, PERK, IRE1α, ATF4, and ATF6 (Hotamisligil & Erbay, 2008; Verfaillie, Garg, & Agostinis, 2013). In addition, IL-8, IL-6, monocyte chemoattractant protein 1, and TNF-α induced by ER stress facilitate inflammation and support the development of TME (Li et al., 2005).

Diet and nutritional constituents may play an important role in governing the development and progression of various types of cancers by effectively managing the chronic inflammatory conditions. This is implicated in the report published by the American Cancer Society in 2011 where it is showed that 30% of cancer cases in developed countries and 20% in developing countries are related to and influenced by diet (Mcguire, 2016). Use of probiotics has been implicated in the modulation of oncogenic responses or helping better management of stress induced by chemotherapies.

3 Mechanism of action of probiotics against cancer

The mechanistic studies that specify the mechanisms by which probiotics induce its anticancer effects are ever evolving and are still being understood. The complexity also arises due to the fact that various strains of probiotics show specific action against cancer. However, it is evident that probiotics influence several oncogenic pathways directly or indirectly through extensive routes (Fig. 12.2). Some of these pathways deserve special mention.

3.1 Modulation of oxidative stress

Chronic inflammation, as discussed in the previous section, is a major contributing factor to carcinogenesis, and oxidative stress is a major precursor that is characterized by an imbalance between ROS and antioxidants present in the body. Interaction between ROS and the cellular molecules such as cellular protein, lipid, and DNA leads to acute and chronic cell injury that possibly perpetuates into cancer.

Probiotics induce their antioxidative effects by several mechanisms. One of the mechanisms is synthesizing metabolites with antioxidant activity, such as glutathione (GSH), butyrate, and folate. Kullisaar et al. (2002) found that the two antioxidative *Lactobacillus fermentum* strains, E-3 and E-18, contained remarkable levels of GSH activities. MIYAIRI 588 strain of *Clostridium butyricum* induces antioxidizes and reduces hepatic oxidative stress in in vivo studies with rats having nonalcoholic fatty liver disease by producing butyrate short-chain fatty acid (SCFA) (Endo, Niioka, Kobayashi, Tanaka, & Watanabe, 2013). Normal cellular processes such as oxidative phosphorylation and cellular metabolism of substances by P450 enzymes induce the formation of ROS and lead to endogenous oxidative stress (Parke & Ioannides, 1990). In vitro studies have reported that LGG and fermented milk containing LGG are capable of scavenging superoxide anion and inhibiting lipid peroxidation reactions (Ahotupa, Saxelin, & Korpela, 1996). *Bifidobacterium longum* (ATCC 15708) and *L. acidophilus* (ATCC 4356) show most of the antioxidative activities. These two probiotic species demonstrate inhibiting activities up to 28%–48% against linoleic acid peroxidation. They also show scavenging activities against free radicals such as *a*-diphenyl-*b*-picrylhydrazyl. Probiotics can also produce antioxidative enzymes and thereby can regulate oxidative

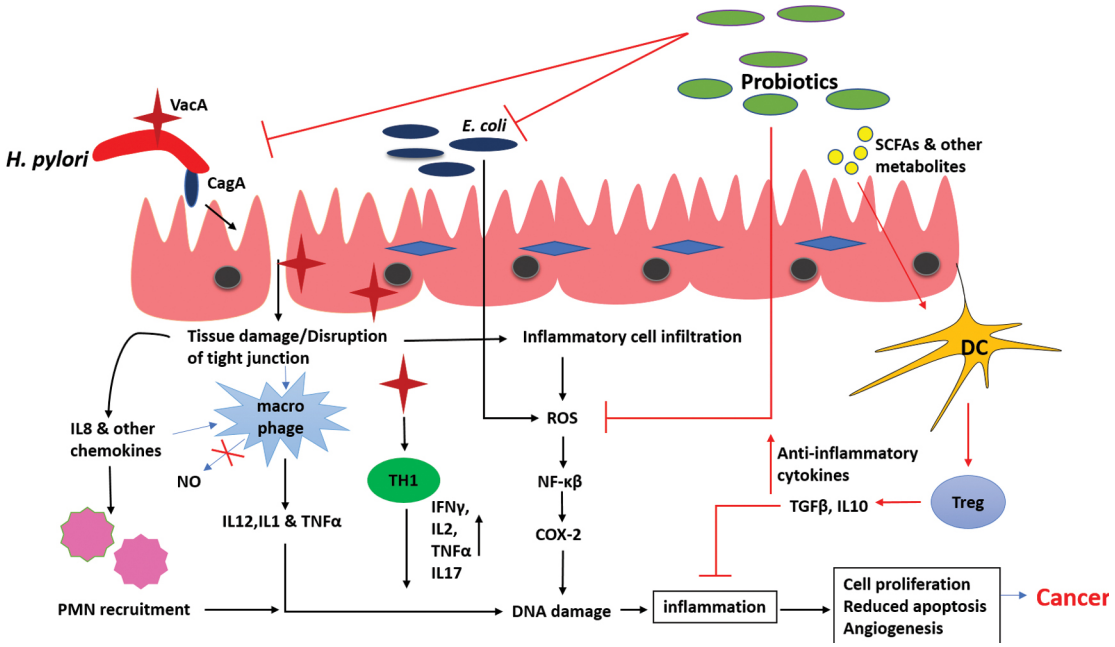


FIGURE 12.2 Probiotics initiating anti-oncogenic signaling.

stress responses. For example, *L. fermentum* E-3 and E-18 were able to express Mn-SOD to resist oxidative stress (Wang, Yi, Yu, Dong, & Qiao, 2009). Consumption of *L. fermentum* increases serum SOD and GPx and enhances hepatic CAT, muscle SOD, and Cu and Zn-SOD (Wang, Liu, Gao, & Hao, 2013). In addition, probiotic bacteria can modulate antioxidant signaling pathways and thus reduces oxidative stress. For example, probiotic bacteria can protect against oxidative stress through regulating the Nrf2-Keap1-antioxidant response element (ARE) pathway (Zhang, 2006). When the concentration of ROS is less, Nrf2 is bound to its cytoplasmic inhibitor Keap1, which suppresses the activity of Nrf2. After activation, Nrf2 translocates into the nucleus and binds to ARE sequences, promoting the transcription of ARE-driven genes such as genes encoding antioxidant enzymes (Klaassen & Reisman, 2010; Maher & Yamamoto, 2010; Srivastava et al., 2011). The extracellular polysaccharide from *Bacillus* sp. strain LBP32 prevented Lipopolysaccharide (LPS)-induced inflammation by inhibiting NF-κB, a transcription factor responsible for inflammatory cytokine secretion (Diao et al., 2014; Gómez-Guzmán et al., 2015). ROS are generated by several enzymatic reactions and recently probiotics have shown modulatory effect on activities of these enzymes, including NADPH oxidase (NOX-1 and NOX-4), cyclo-oxygenase (COX-2), the cytochrome P450 (CYP) enzymes such as CYP1A1, CYP1A2, and CYP1B1 (Wang et al., 2017; Brzozowski et al., 2006; Patel et al., 2016; Bondy & Naderi, 1994; Zangar, Davydov, & Verma, 2004; Matuskova et al., 2011).

3.2 Effects on carcinogen/genotoxic compounds

Lactobacillus (LAB) and *Bifidobacterium* strains can bind and degrade potential carcinogens that are produced during cooking of meat (heterocyclic aromatic amines), fungal toxin such as aflatoxin B1, and food contaminant AF2, and others such as mycotoxins, polycyclic aromatic hydrocarbons, and phthalic acid esters nitrosamines (Burns & Rowland, 2004; Nowak, Kuberski, & Libudzisz, 2014; Duangjitcharoen, Kantachote, Prasitpuripreecha, Peerajan, & Chaiyasut, 2014; Lili, Junyan, Hongfei, Baoqing, & Bolin, 2018; Challa, Rao, Chawan, & Shackelford, 1997). In vitro studies have reported the effects of *B. longum*, and LAB in the prevention of colon cancer incidence. These probiotic bacteria induce the activities of GSH S-transferase enzyme that detoxifies toxic metabolites and carcinogens in colon and enhances O6-methylguanine in colonic mucosal DNA and suppressed azoxymethane (AOM)-induced colonic aberrant crypt foci (ACF) (Arimochi, Kinouchi, Kataoka, Kuwahara, & Ohnishi, 1997; Uccello et al., 2012).

3.3 Effects on bacterial enzymatic activity

One of the main mechanisms employed by probiotic bacteria to exhibit its health benefits is to maintain natural intestinal microflora both qualitatively and quantitatively thereby controlling their metabolic activities. Glucuronide conjugation is an important enzymatic process which is responsible for the removal of toxins and carcinogenic compounds in the liver. Some

putrefactive microorganisms that are part of normal gut microflora such as *Escherichia coli* and *Clostridium perfringens* deconjugate these glucuronides in the intestine by β-glucuronidase enzyme producing aglycones that are potentially carcinogenic substances and may induce colon cancer (Reddy, Mangat, Weisburger, & Wynder, 1977; Hill, 1975). In addition, some other bacterial enzymes mainly azoreductase and nitroreductase are also responsible for the liberation of carcinogenic substances in intestine; however, probiotics have demonstrated to reduce the activity of these enzymes that substantially reduced the chances of having cancer (Goldin & Gorbach, 1976, 1984a,b). Oral supplementation of *L. acidophilus* for 10 days reduces activity of three enzymes (β-glucuronidase, nitroreductase, and azoreductase) (Marteau et al., 1990). Consumption of fermented dairy product containing *L. acidophilus*, *B. bifidum*, and mesophilic cultures (*Streptococcus lactis* and *Streptococcus cremoris*) for 3 weeks decreases the activity of nitroreductase, while β-glucuronidase and azoreductase activities remain unchanged (Marteau, Pochart, & Flourié, 1990). However, in human studies the results have not been particularly encouraging and limited success has been observed in reducing effects of probiotics on these enzymes.

3.4 Immunomodulatory functions against cancer

Immune system components such as T cells, B cells, antigen presenting cells, natural killer cells (NK cells), and inflammatory cytokines exhibit antitumor activities and ameliorate tumor development and progression (Sekine et al., 1985). Recent studies have reported the modulatory effects of probiotic bacteria on immune system. Almost 80% of the immune cells are related to intestinal mucosa and probiotic bacteria have the ability to adhere intestinal epithelium, interact with immune cells, and enhance mucosal and systemic immune response, thus inducing its anticancerous effects (Jungersen et al., 2014; Maccaferri et al., 2012; Matsuzaki, Yokokura, & Mutai, 1988). *Lactobacillus casei Shirota* (LcS) has shown antitumor effects both in vitro and in vivo studies mainly by modulating host immune system. Intrapleural administration of LcS has shown powerful antitumor and antimetastatic effects in chemically induced cancer rats by inducing the expression of several cytokines (Matsuzaki, 1998; Malaguarnera et al., 2012). After ingestion, LcS is incorporated into payers' patches where macrophages or DCs phagocytose LcS and produce several cytokines, especially TNF-α. DCs and type 1 helper T cells (Th1) are also stimulated by LcS and may contribute in antitumor effects (Foligne et al., 2007; Yasui, Shida, Matsuzaki, & Yokokura, 1999). Administration of *L. acidophilus*, *L. casei* YIT9029, and *B. longum* HY8001 for 4 weeks increased the survival rate of mice injected with tumor cells that was found to be mediated by increase in T cells, NK cells and MHC class II+ cells, and CD4–CD8 + T cells (Kumar, Sastry, Polaki, & Mishra, 2015; Lee et al., 2004). In vivo studies with mice revealed that consumption of *L. acidophilus* (10^9–10^{11} CFU/mL) reduces tumor size and delays colon cancer progression modulating gene expression pathway(s) in colonocytes (Ivanov & Honda, 2012; Kumar, Sastry, Polaki, & Mishra, 2015). Probiotics are also known to synthesize and secrete various metabolites mainly SCFAs. These metabolites subsequently act as signaling molecules and modulate the secretion of prostaglandins and inflammatory cytokines and thus result in lower chances of cancer progression (Delcenserie et al., 2008; Ivanov & Honda, 2012).

3.5 Effects of probiotics on tumor microenvironment

Initial stages of tumor development are highly dependent on the interaction between tumoral and nontransformed cells and the surrounding environment they create. This environment also known as tumor microenvironment consists of cells of the immune system, the tumor vasculature, and lymphatics, as well as the fibroblasts, pericytes, myofibroblasts, extracellular matrix components, and adipocytes (Wang, Zhao, & Zhang, 2017). TME facilitates tumor growth and progression. Recent studies have shown that probiotic *L. casei* YIT9018 exert antitumoral effects by modulating microenvironment. Administration of milk fermented by *L. casei* CRL 431 inhibited tumor growth with significant reduction in the level of tumor vascularity, extravasation of tumor cells, and lung metastasis. Aforementioned effects are mainly driven by the alterations in the immune responses such as a decreased rate of infiltrating macrophages in both the tumor and the lungs and an increased antitumor response associated to CD8+ and CD4+ lymphocytes (Aragón, Carino, & Perdigón, 2015). Administration of *L. casei* YIT9018 has also been shown to elicit a high level of IL-2 and IFN-γ expression that acts against tumor cells in TME and also downregulated IL-1β and vascular endothelial growth factor (VEGF) which is an important angiogenic factor that promotes tumor invasiveness (Kumar et al., 2010). Effects of various probiotic strains on TME is less established but it is very compelling and further research will contribute more on this specific area of research.

3.6 Effects of probiotics on apoptosis

Apoptosis is a form of genetically programmed cell death that occurs in every multicellular organism, and any impairment of this process either due to over expression of oncogenes or suppressed levels of tumor suppressor genes results in an

uncontrolled accumulation of malignant cells that subsequently leads to cancer. Control over apoptosis process is also very important as uncontrolled, excessive amount of apoptosis hinder cellular homeostasis. Consumption of probiotics might play an important role in maintaining cellular growth and senescence as several strains of probiotic bacteria have shown both antiapoptotic and proapoptotic characteristics. In vitro studies with either human or mouse colon cells cocultured with LGG induce antiapoptotic Akt/protein kinase and inhibit proapoptotic p38/mitogen-activated protein kinase by TNF, IL-1α, or IFN-γ (Kumar et al., 2010; Le Leu et al., 2005). A synbiotic combination of resistant starch and *Bifidobacterium lactis* induces apoptosis in carcinogen-driven colorectal cancer (CRC) model in rodents and also exhibits some controlling effects in differentiation process of tumor cells in CRC (Le Leu et al., 2005; Le Leu, Hu, Brown, Woodman, & Young, 2010). Live *L. casei* has exhibited inhibitory effects on tumor growth in both murine (CT26) and human (HT29) colon carcinoma cell lines by inducing apoptotic cell death upregulating TNF-related apoptosis-inducing ligand (Tiptiri-Kourpeti et al., 2016).

4 In vitro studies of probiotics on cancer

In vitro studies are mostly conducted on colonic cancer cells or GC cells and their cell lines; these studies have shown antiproliferative or proapoptotic activities of various probiotic bacteria. The anticancer activities of probiotic bacteria are mainly mediated by components from their cell wall or soluble compounds produced during fermentation (Biffi, Coradini, Larsen, Riva, & Di Fronzo, 1997; Bogdanov et al., 1975). In addition, heat-killed probiotic bacteria, secreted metabolites, and their cytoplasmic fractions have also been shown anticancer effects. Another metabolic product exopolysaccharides (EPS) have recently been shown health benefits, including antitumor activities (Choi et al., 2006; Sekine et al., 1985). Two cellular proteins, Beclin-1 and GRP78, play an important role in autophagy-related cell death pathways and cell-bound EPS (cb-EPS) isolated from *L. acidophilus* 606 regulate their activities (Kim, Oh, Yun, Oh, & Kim, 2010). In one in vitro study with HT-29 colon cancer cells it has reported that cb-EPS induces its antitumorigenic effects by directly regulating autophagic cell death components Beclin-1, GRP78 (Kim et al., 2010). Among various LAB strains, LGG shows prominent anticancer activities. Cytoplasmic extract from LGG homogenate inhibits cell growth and polyamine synthesis in both HGC-27 and DLD-1 human cancer cell line (Linsalata et al., 2010; Orlando et al., 2012). Neoplastic growth is one of the characteristics of cancer; however, it depends on the rate of cell proliferation and apoptosis. Viable or heat-killed *Lactobacillus paracasei* IMPC2.1 and LGG when cocultured with HGC-27 and DLD-1 cells show antitumor activities by inducing apoptosis and inhibiting cell proliferation in in vitro studies (Orlando et al., 2012). Studies conducted by Lee et al. have reported that cytoplasmic fractions of *L. acidophilus*, *L. casei*, and *B. longum* showed significant antitumor activities in some cancer cell lines (Lee et al., 2004). In vitro studies on breast cancer cell line (MCF7) either with freeze–dried *L. acidophilus* La-05, *L. casei* spp. *paracasei* Lc-01, or live *L. acidophilus* and *L. casei* show antiproliferative effects (Abedin-Do, Taherian-Esfahani, & Ghafouri-Fard, 2015). Heat-killed cells of *L. casei* ATCC 393 and *L. acidophilus* 606 have also shown to inhibit the proliferation of human pancreatic cancer (PC) cell line (PANC-1) and HT29 and HeLa cancer cells by producing soluble polysaccharide. α-Lactalbumin secreted from LAB forms complexes with cellular oleic acids and shows lethal activities against cancer cells (Hassan, 2019; Liu & Pan, 2010). Heat-killed cells and cytoplasmic fractions of different species of LAB and *Bifidobacteria* were studied and reported a high antiproliferative and antioxidant effects, for example, heat-killed cells of these bacteria reduce the viability of human breast adenocarcinoma cells to 46.3% and cytoplasmic fraction of the bacteria reduces viability to 31.09% on breast cancer cells (MCF-7) (Hassan et al., 2016; Arimochi et al., 1997). In another study, it has been observed that the proliferation of three human colon cancer cell lines, including HT-29, SW 480, and Caco-2, was inhibited by the probiotic product *Bifidobacterium adolescentis* SPM0212 (Kim et al., 2008b). Not only this probiotic product but other products and strains such as *Bacillus polyfermenticus*, *L. acidophilus* 606, LGG/Bb1, and LGG/*Bifidobacterium animalis* subsp. *lactis* induce antitumor effects on various cancer cell lines (Stein et al., 2012; Soltan Dallal, Mojarrad, & Baghbani, 2015; Arimochi, Kinouchi, Kataoka, Kuwahara, & Ohnishi, 1997; Ma et al., 2010; Orlando et al., 2012). Cancer cell invasion and metastasis, which are the primary mechanisms of cancer progression, are achieved by altering in extracellular matrix and basement membranes. These activities of cancer cells are mediated by proteolytic enzymes, including serine proteinase and metalloproteinase, their respective receptors (McMahon and Kwaan, 2015). Recent investigation on cancers such as breast, colon, gastric, prostate, and lung has reported an unimpeded expression of urokinase plasminogen activator (uPA) and uPA receptor (uPAR) (Ding et al., 2016; Mazar, 2001). When human gastric adenocarcinoma epithelial cell line (AGS) cells were treated with *Lactobacillus reuteri*, it inhibits cancer cell proliferation in dose-dependent manner by regulating uPA and uPAR gene expression (Kim et al., 2005; Rasouli, Ghadimi-Darsajini, & Nekouian, 2017). Many other in vitro studies have also reported the potential therapeutic approaches of various probiotic bacteria in the cancer treatment. Table 12.1 shows some of the prominent studies that depict in vitro studies with different probiotic strains.

TABLE 12.1 In vitro studies.

Probiotic strain	Cancer type/Cell line used	Effects	References
Bifidobacterium adolescentis SPM012	HT-29, SW 480, and Caco-2 (in vitro)	Inhibited harmful fecal enzymes, including α-glucuronidase, α-glucosidase, tryptophanase, and urease	(Kim et al., 2008b)
Lactobacillus Bulgaricus	1,2-Dimethylhydrazine (DMH)-inhibited colon cancer (in vivo)	Inhibited tumor progression and promotion by modulating the immune response and stimulating cellular apoptosis	(de Moreno de Leblanc and Perdigón, 2004)
Bacillus Polyfermenticus	HT-29 (human gastric cancer), DLD-1, or Caco-2 human colon cancer cells	Reduced ErbB2 and ErbB3 protein expression as well as mRNA levels	(Iyer et al., 2008; Ma et al., 2010)
Lactobacillus Rhamnosus GG and *Lactobacillus Paracasei* IMPC2.1	HGC-27 gastric (human gastric cancer) and DLD-1 (human colorectal adenocarcinoma cells) colon cell lines	Induced apoptosis and inhibited the cancer growth	(Orlando et al., 2012)
Lactobacillus reuteri ATCC 6475	Human chronic myeloid leukemia-derived cells (KBM-5)	TNF-induced apoptosis and suppressed cell proliferation and antiapoptotic proteins	Iyer et al. (2008)
Bifidobacterium infantis, *Bifidobacterium bifidum*, *Bifidobacterium animalis*	MCF7 breast cancer cell lines	Bioactive compounds having anticancer activity	(Arimochi et al., 1997)
B. polyfermenticus KU3	Cervix and breast cancer	Cytotoxicity against cancer cells	Lee et al. (2015b)
Lactococcus lactis NK34	Lung and breast cancer	Inhibition of proliferation of cancer cells	(Han et al., 2015)
Lactobacillus fermentum	Caco-2 and SW-480	Inhibition of cell proliferation and induction of apoptosis. The suppression is associated with SCFA production	(Kahouli et al., 2015b; Kahouli, Malhotra, Alaoui-Jamali, & Prakash, 2015a)
Lactobacillus acidophilus, *Lactobacillus casei*	Colorectal tumor cells Caco-2	Induction of necrosis and apoptosis. Reduction of migration and invasion ability	(Soltan Dallal et al., 2015)
Lactobacillus crispatus and *L. rhamnosus*	Cervical and colon cancer (HeLa and HT-29 cell lines)	Cytotoxic effects	(Nouri et al., 2016)
Pediococcus pentosaceus FP3, *Lactobacillus salivarius* FP25/FP35, *Enterococcus faecium* FP51	Colon cancer cells (Caco-2)	Inhibition of cell proliferation activation of apoptosis	(Thirabunyanon and Hongwittayakorn, 2013)

[a]Iyer C, Kosters A, Sethi G et al. (2008)

5 In vivo studies of probiotics on cancer

In vivo studies are conducted mainly by creating animal model experiments using rats and mice to elucidate more about the anticancer effects of probiotics and recently some encouraging results have also been obtained. Colonic cancer in rats and mice induced by carcinogen was significantly inhibited by a combination of *L. acidophilus* and *B. bifidum* (Park, Jeon, Park, & Paik, 2007; Lee, Park, Park, & Paik, 2007; Chang, Shim, Cha, Reaney, & Chee, 2012; Kumar et al., 2012; Asha & Gayathri, 2012; Mohania, Kansal, Kruzliak, & Kumari, 2014; Le Leu, Hu, Brown, Woodman, & Young, 2010; McIntosh et al., 1999). In another experimental model, the incidence of AOM-induced colonic ACF and colon cancer was significantly decreased by orally administered probiotic combinations (Nakanishi, Kataoka, Kuwahara, & Ohnishi, 2003; Yamazaki et al., 2000; Femia et al., 2002; Marotta et al., 2003; Zhang et al., 2012). In vivo studies also reported that probiotics can alleviate dysbacteriosis, ameliorate intestinal inflammation, and decrease liver tumor growth thus reducing the chances of the development of hepatocellular carcinoma (HCC) (Zhang, Yu, & Yang, 2012). Incidence of oral cancer in rats induced by 4-nitroquinoline 1-oxide can also be inhibited by *L. salivarius* (Zhang et al., 2013). Several studies have reported the correlation between body's inflammatory response and the development of cancer. *H. pylori* as described before acts as

a carcinogen and induces cancer development in stomach. Inflammatory responses induced by *H. pylori* play the central role in stomach cancer. One way to control *H. pylori*–induced GC is to modulate immune system as well as inflammatory responses. In various experimental models, it has been shown that probiotic *L. acidophilus*, for example, induces IL-12 production in BALB/c mice which, in turn, modulate immune responses with the help of NK cells (Soltan Dallal et al., 2012). In another experiment, *L. casei* spp. *casei* ATCC 39392 was administered orally to 6–8-week-old female BALB/c mice for 2 weeks and then tumor cells were transplanted. After transplantation of tumor cells, *L. casei* spp. *casei* ATCC 39392 administration was continued for another 3 weeks with 3-day intervals between the weeks. At the end of this experiment, an increasing number of immune modulators such as IL-12, IFN-γ, and NK cells were observed (de Moreno de LeBlanc, Matar, LeBlanc, & Perdigón, 2005). Thus probiotic bacteria could counter tumor genesis by modulating immune system components. Another study with probiotic *Lactobacillus helveticus* R389 reported that this probiotic bacterium can delay tumor formation by decreasing IL-6 and increasing the expression of IL-10, and also inducing apoptosis in tumor cells (Sheikhpour et al., 2018). In addition, IL-10 induced by probiotic *L. helveticus* R389 modulates NF-κB translocation pathway by downregulating VEGF, IL-1b, TNF-α, IL-6, and MMP-9 expression (Li et al., 2016). In a very recent in vivo study, a new probiotic mixture, Prohep, which is composed of LGG, viable *E. coli* Nissle 1917 (EcN), and heat-inactivated VSL#3 (1:1:1) was used against HCC in mice. Th17 produces IL-17 in the TME that has linked to HCC growth and angiogenesis. Prohep was effective in reducing HCC growth in mice and this antitumor function offered by Prohep was likely to associate with modulation of the gut microbiota and inducing the secretion of antiinflammatory cytokine such as IL-10 and suppressing Th17 cell differentiation in gut (Cheng, Guan, & Chen, 2019). In addition, this reduced Th17 cells from gut and IL-17 results reduced angiogenesis in liver tumor and subsequently suppressed the tumor growth (Li et al., 2016). Table 12.2 presents a detailed account of some in vivo studies that have shown significant reduction in cancer properties.

TABLE 12.2 In vivo studies.

Probiotic strain	Cancer type/Cell line used	Effects	References
Bifidobacterium spp.	Melanoma (TAC mice)	Tumor size decreased	(Sivan et al., 2015)
Lactobacillus plantarum LS/07	Breast cancer (SD rats)	Cell proliferation marker was reduced	(Kassayová et al., 2016)
Probiotics mixture	Liver cancer (C57BL6/N mice)	Reduced tumor size and downregulated angiogenic factors	(Li et al., 2016)
L. plantarum BFLP28	Sarcoma (BALB/c mice)	Suppression of tumor growth, together with modulation of immune responses including cytokine production and CD3+ T-cell population	(Shin et al., 2016)
Lactobacillus acidophilus, *B. bifidum*, and *Bifidobacteria infantum*	Colon cancer (Sprague-Dawley rats)	Reduction of tumor incidence and volume by probiotics such suppression was associated with the improvement of intestinal integrity and mediated by Toll-like receptor (TLR)2	(Kuugbee et al., 2016)
Lactobacillus rhamnosus GG CGMCC 1.2134	Colon cancer (Sprague-Dawley rats)	Reduced the expression of β-catenin and the inflammatory proteins NFκB-p65, COX-2, and TNFα; the antiapoptotic protein Bcl-2 but increased the expression of the proapoptotic proteins Bax, casp3, and p53	(Gamallat et al., 2016)
L. plantarum (AdF10) and *L. rhamnosus* GG	Colon cancer (Sprague-Dawley rats)	Reduction of tumor incidence, multiplicity, and volume; decrease in cyclooxygenase (COX)-2 protein expression and total sialic acid (TSA) level	(Walia et al., 2015)
Lactobacillus casei BL23	Colorectal cancer	Modulation of regulatory T cells	(Lenoir et al., 2016)
Prohep (probiotic mixture)	Hepatocellular carcinoma (HCC)	Reduction in tumor size and growth	(Li et al., 2016)
L. plantarum	Colon cancer (BALB/c mice)	A decreased tumor incidence, cell cycle arrest induction of apoptosis	(Lee et al., 2015a)

6 Probiotics and gastrointestinal (GI) cancer

GI cancer refers to a group of cancer that affects various parts of digestive system that includes GC, CRC, HCC, esophageal cancer, and PC. Aging, exponential growth of the population, obesity, changing lifestyle and high prevalence of *H. pylori*, hepatitis B virus and hepatitis C virus, and several other factors are responsible for the development of GI cancers. However, in India the incidence of GI cancer cases is relatively low and accounts fifth most common cancer among men and sixth most common in women (Rao & Ganesh, 1998; Dikshit et al., 2012). Anticancer properties of probiotics which we have discussed in previous sections make it a suitable candidate to treat or prevent the onset of various GI cancers. Studies have already reported the relative efficacy of probiotics against PC, GC, CRC, and others.

6.1 Probiotics against colorectal cancer (CRC)

Both men and women suffer equally from the incidence of CRC, and in the last 20–30 years the rate of CRC has not been decreased. Evidences from wide range of reports suggest that diet as well as colonic microflora is involved in the etiology of CRC (Salminen et al., 1998; Glade, 1999). Several studies have reported that probiotics can modulate the gut microflora and their metabolism and selectively stimulate the growth and/or activity of one or a limited number of bacteria in the colon that have the potential to improve host health (Salminen et al., 1998; Zhong, Zhang, & Covasa, 2014). Thus using probiotics as a diet intervention and natural bioactive supplement to reduce the risks of colon cancer could be an effective therapeutic approach. Studies have shown that the presence of *Bifidobacterium* inhibits the colon carcinogenesis by lowering the pH of the colon, which results in inhibition of *E. coli* and clostridia the growth (Singh et al., 1997). Another study has reported that *Bifidobacterium* affects the activities of hepatic cytochrome P4501A2 and acetyltransferase-2 (NAT-2) which catalyzes *N*-oxidation followed by *O*-acetylation steps in food-borne heterocyclic amines which act as a colon carcinogen (Singh et al., 1997; Lang et al., 1994). Another probiotic bacteria *B. longum* reduces the activity of ornithine decarboxylase which is involved in cellular proliferation, differentiation, and macromolecular synthesis and is responsible for colon adenomas and carcinomas (Moorehead, Hoper, & McKelvey, 1987). Some commensal microorganisms such as *Bacteroides fragilis* and *E. coli* are capable of metabolizing xenobiotics and producing carcinogenic compounds in this process thus increasing the chances of CRC. Probiotic bacteria biotransform procarcinogens and carcinogens into less toxic substances and also inhibit CRC-promoting bacteria by secreting antimicrobial substances, metabolites such as SCFAs (Louis, Hold, & Flint, 2014; Ostaff, Stange, & Wehkamp, 2013). In addition, various studies have reported the effectiveness of probiotics in preventing CRC through other mechanisms, namely, immune modulation (Kahouli, Tomaro-Duchesneau, & Prakash, 2013; Chong, 2014; Smits et al., 2005) and inducing apoptosis (Uccello et al., 2012; Daniluk, 2012).

6.2 Probiotics against gastric cancer (GC)

Gastric adenocarcinoma and lymphoma in humans are mainly caused by pathogenic *H. pylori* that is classified as class 1 carcinogen. Functional attributes mainly caused by probiotics on GC are focused primarily on eliminating or combating against this pathogen. After discovering the role of *H. pylori* in development and progression of GC, it is evident that microbes play a great amount role in its pathophysiology. A strong corelation between *H. pylori* infection and GC is revealed by epidemiological studies. More than half of the world's population is infected by *H. pylori* however only 5%–10% develops ulcers providing deep insight that there are other factors that determine whether the infection will be produced or not. In addition, nonsteroidal antiinflammatory drugs (NSAIDs), low-dose aspirin, smoking, excessive alcohol use, and emotional stress are some other important causes of ulcers other than *H. pylori* infection. *H. pylori* is responsible for majority of the peptic ulcer incidence worldwide. *H. pylori* induces secretion of epithelium-derived inflammatory cytokines mainly IL-8 and IL-1β and causes inflammation in gastric mucosa (Noach et al., 1994). Neutrophils and macrophages are attracted toward this inflamed mucosa and release lysosomal enzymes, leukotrienes, and ROS which destroy mucosal defenses and induce immune-pathogenetic process of ulcer formation. For survival in acidic gastric environment, *H. pylori* produces urease, which catalyzes the formation of ammonia, to create an alkaline environment. On the other hand, higher concentration of ammonia produces ammonium chloride which then combines with bacterial phospholipases A and C and diminishes the mucosal integrity. *H. pylori* also expresses adhesion proteins such as blood group antigen adhesin or outer inflammatory protein adhesin for its attachment to gastric epithelium. In addition, genome pathogenic island encodes various virulence factors such as CagA and PicB that facilitate gastric mucosal inflammation, cell infiltration, and gastric epithelial injury (Shiotani & Graham, 2002; Yamaoka & Graham, 2014; Datta De and Roychoudhury, 2015; Yamaoka and Graham, 2014). There is a lack of knowledge of how *H. pylori* induces various types of gastroduodenal mucosal lesions. Cytokines secreted due to inflammation inhibits parietal cell secretion (Zaki et al., 2013; Beales and Calam, 2001), or

H. pylori–secreted products directly effect on the H + /K+ ATPase α-subunit, or inhibition of gastrin (Zaki et al., 2013) leads to either hypochlorhydria or hyperchlorhydria (Meyer-Rosberg et al., 1996).

Several animal models have reported the inhibitory effects of probiotic strains against *H. pylori*. Probiotics have demonstrated stimulating effects on mucosal immune system. Probiotics activate mucosal resident macrophages and increase their antigen presentation and maintain cytokine homeostasis. *H. pylori* infected children when supplemented with probiotics-containing yogurt, a higher level of serum IgA, and reduced serum IL-6 was observed (Li et al., 2014). In addition, probiotics may also exert its effects through nonimmune mechanisms such as antagonistic and/or competitive effects against pathogenic organisms. In vitro studies with LAB strains such as *Lactobacillus gasseri Chen*, *Lactobacillus plantarum*, *L. acidophilus*, *Lactobacillus johnsonii*, and *L. salivarius* subsp. *salicinius* inhibit *H. pylori* adherence to gastric epithelial cells and gastric adenocarcinoma cell line AGS (Chen et al., 2012). Probiotics are also capable of producing antioxidants and antimicrobial substances, can enhance intestinal barrier function by modulating tight junctions between epithelium, stimulate mucin production, and can maintain local pH balance and many other undefined functions (Del Giudice, Malfertheiner, & Rappuoli, 2009; Yang & Sheu, 2012). *L. johnsonii* and *L. salivarius* were also able to diminish increase of IL-8 production, reduction of bacterial load, and of gastric inflammation which is induced by *H. pylori* in AGS cells (Hsieh et al., 2012). Conjugated linoleic acids synthesized by *L. acidophilus* modulate NF-κB pathway and inhibit *H. pylori*–induced inflammations (Kim et al., 2008a; Yang, Chuang, Yang, Lu, & Sheu, 2012; Hwang et al., 2012).

7 Afterword

Currently available standard cancer drugs are highly effective in suppressing cancer cells and their progression. However, these drugs are expensive, nonspecific, and induce toxic effects on other noncancerous cells. Probiotic therapy has shown promise, albeit preliminary, in preclinical studies against cancer, and has generated enough interest as an alternative biotherapeutic agent. Overall an increasing number of scientific studies have highlighted the important role of probiotics in maintaining intestinal homeostasis and projected the subsequent antiinflammatory and immune modulatory effects to the development of successful cancer therapy. However, future research findings and in-depth studies are necessary to validate all anticancer effects before fully establishing probiotics as therapeutics. Clinical tests of the interesting strains will pave the way for the use of these probiotics as potential drug-delivery vectors and noninvasive cancer treatment options in humans.

References

Abedin-Do, A, Taherian-Esfahani, Z, Ghafouri-Fard, S, et al. (2015). Immunomodulatory effects of Lactobacillus strains: emphasis on their effects on cancer cells. *Immunotherapy*, *7*, 1307–1329.

Ahotupa, M., Saxelin, M., & Korpela, R. (1996). Antioxidative properties of *Lactobacillus* GG. *Nutrition Today*, *31*, 51S–52S.

Aragón F, Carino S, Perdigón G et al. (2015) Inhibition of Growth and Metastasis of Breast Cancer in Mice by Milk Fermented With Lactobacillus casei CRL 431. Journal of immunotherapy (Hagerstown, Md. : 1997) 38:185–196

Arimochi, H., Kinouchi, T., Kataoka, K., Kuwahara, T., & Ohnishi, Y. (1997). Effect of intestinal bacteria on formation of azoxymethane-induced aberrant crypt foci in the rat colon. *Biochemical and Biophysical Research Communications*, *238*, 753–757.

Asha, & Gayathri, D. (2012). Synergistic impact of *Lactobacillus fermentum*, *Lactobacillus plantarum* and vincristine on 1,2-dimethylhydrazine-induced colorectal carcinogenesis in mice. *Experimental and Therapeutic Medicine*, *3*, 1049–1054.

Bazuro, GE, Torino, F, Gasparini, G, et al. (2008). Chemoprevention in gastrointestinal adenocarcinoma: for few but not for all? *Minerva Gastroenterol Dietol*, *54*, 429–444.

Beales, I. L., & Calam, J. (2001). Inhibition of carbachol stimulated acid secretion by interleukin 1beta in rabbit parietal cells requires protein kinase C. *Gut*, *48*, 782–789.

Biffi, A., Coradini, D., Larsen, R., Riva, L., & Di Fronzo, G. (1997). Antiproliferative effect of fermented milk on the growth of a human breast cancer cell line. *Nutrition and Cancer*, *28*, 93–99.

Blaser, MJ (2014). The microbiome revolution. *J Clin Invest*, *124*, 4162–4165.

Bogdanov, I. G., Dalev, P. G., Gurevich, A. I., Kolosov, M. N., Malékova, V. P., Plemyannikova, L. A., et al. (1975). Antitumour glycopeptides from *Lactobacillus bulgaricus* cell wall. *FEBS Letters*, *57*, 259–261.

Boland, M. L., Chourasia, A. H., & Macleod, K. F. (2013). Mitochondrial dysfunction in cancer. *Frontiers in Oncology*, *3*, 292.

Bondy, S. C., & Naderi, S. (1994). Contribution of hepatic cytochrome P450 systems to the generation of reactive oxygen species. *Biochemical Pharmacology*, *48*, 155–159.

Brzozowski, T., Konturek, P. C., Mierzwa, M., Drozdowicz, D., Bielanski, W., Kwiecien, S., et al. (2006). Effect of probiotics and triple eradication therapy on the cyclooxygenase (COX)-2 expression, apoptosis, and functional gastric mucosal impairment in *Helicobacter pylori*-infected *Mongolian gerbils*. *Helicobacter*, *11*, 10–20.

Burns, A. J., & Rowland, I. R. (2004). Antigenotoxicity of probiotics and prebiotics on faecal water-induced DNA damage in human colon adenocarcinoma cells. *Mutation Research*, *551*, 233–243.

Challa, A., Rao, D. R., Chawan, C. B., & Shackelford, L. (1997). *Bifidobacterium longum* and lactulose suppress azoxymethane-induced colonic aberrant crypt foci in rats. *Carcinogenesis*, *18*, 517–521.

Chang, J. H., Shim, Y. Y., Cha, S. K., Reaney, M., & Chee, K. M. (2012). Effect of *Lactobacillus acidophilus* KFRI342 on the development of chemically induced precancerous growths in the rat colon. *Journal of Medical Microbiology*, *61*, 361–368.

Chen, X., Liu, X. M., Tian, F., Zhang, Q., Zhang, H. P., Zhang, H., et al. (2012). Antagonistic activities of lactobacilli against *Helicobacter pylori* growth and infection in human gastric epithelial cells. *Journal of Food Science*, *77*, M9–M14.

Cheng, H, Guan, X, Chen, D, et al. (2019). The Th17/Treg Cell Balance: A Gut Microbiota-Modulated Story. *Microorganisms*, *7*, 583.

Choi, SS, Kim, Y, Han, KS, et al. (2006). Effects of Lactobacillus strains on cancer cell proliferation and oxidative stress in vitro. *Letters in Applied Microbiology*, *42*, 452–458.

Daniluk, U. (2012). Probiotics, the new approach for cancer prevention and/or potentialization of anti-cancer treatment? *Journal of Clinical & Experimental Oncology*, *1*, 2.

Datta De, D., & Roychoudhury, S. (2015). To be or not to be: The host genetic factor and beyond in *Helicobacter pylori* mediated gastro-duodenal diseases. *World Journal of Gastroenterology*, *21*, 2883–2895.

de Moreno de Leblanc, A., & Perdigón, G. (2004). Yogurt feeding inhibits promotion and progression of experimental colorectal cancer. *Medical Science Monitor*, *10*, BR96–BR104.

de Moreno de LeBlanc, A., Matar, C., LeBlanc, N., & Perdigón, G. (2005). Effects of milk fermented by *Lactobacillus helveticus* R389 on a murine breast cancer model. *Breast Cancer Research*, *7*, R477–R486.

Del Giudice, G., Malfertheiner, P., & Rappuoli, R. (2009). Development of vaccines against *Helicobacter pylori*. *Expert Review of Vaccines*, *8*, 1037–1049.

Delcenserie, V., Martel, D., Lamoureux, M., Amiot, J., Boutin, Y., & Roy, D. (2008). Immunomodulatory effects of probiotics in the intestinal tract. *Current Issues in Molecular Biology*, *10*, 37–54.

Diao, Y., Xin, Y., Zhou, Y., Li, N., Pan, X., Qi, S., et al. (2014). Extracellular polysaccharide from *Bacillus* sp. strain LBP32 prevents LPS-induced inflammation in RAW 264.7 macrophages by inhibiting NF-κB and MAPKs activation and ROS production. *International Immunopharmacology*, *18*, 12–19.

Dikshit, R., Gupta, P. C., Ramasundarahettige, C., Gajalakshmi, V., Aleksandrowicz, L., & Badwe, R. Million Death Study Collaborators. (2012). Cancer mortality in India: A nationally representative survey The. *Lancet*, 1807–1816.

Ding, Y., Zhang, H., Lu, A., Zhou, Z., Zhong, M., Shen, D., et al. (2016). Effect of urokinase-type plasminogen activator system in gastric cancer with peritoneal metastasis. *Oncology Letters*, *11*, 4208–4216.

Duangjitcharoen, Y., Kantachote, D., Prasitpuripreecha, C., Peerajan, S., & Chaiyasut, C. (2014). Selection and characterization of probiotic lactic acid bacteria with heterocyclic amine binding and nitrosamine degradation properties. *Journal of Applied Pharmaceutical Science*, *4*, 14–23.

Endo, H., Niioka, M., Kobayashi, N., Tanaka, M., & Watanabe, T. (2013). Butyrate-producing probiotics reduce nonalcoholic fatty liver disease progression in rats: New insight into the probiotics for the gut-liver axis. *PLoS One*, *8*, e63388.

Femia, A. P., Luceri, C., Dolara, P., Giannini, A., Biggeri, A., Salvadori, M., et al. (2002). Antitumorigenic activity of the prebiotic inulin enriched with oligofructose in combination with the probiotics *Lactobacillus rhamnosus* and *Bifidobacterium lactis* on azoxymethane-induced colon carcinogenesis in rats. *Carcinogenesis*, *23*, 1953–1960.

Foligne, B., Zoumpopoulou, G., Dewulf, J., Ben Younes, A., Chareyre, F., Sirard, J. C., et al. (2007). A key role of dendritic cells in probiotic functionality. *PLoS One*, *2*, e313.

Gómez-Guzmán, M., Toral, M., Romero, M., Jiménez, R., Galindo, P., Sánchez, M., et al. (2015). Antihypertensive effects of probiotics *Lactobacillus* strains in spontaneously hypertensive rats. *Molecular Nutrition & Food Research*, *59*, 2326–2336.

Gamallat, Y., Meyiah, A., Kuugbee, E. D., Hago, A. M., Chiwala, G., Awadasseid, A., et al. (2016). *Lactobacillus rhamnosus* induced epithelial cell apoptosis, ameliorates inflammation and prevents colon cancer development in an animal model. *Biomedicine & Pharmacotherapy*, *83*, 536–541.

Garrett, W. S. (2015). Cancer and the microbiota. *Science (New York, NY)*, *348*, 80–86.

Glade, M. J. (1999). Food, nutrition, and the prevention of cancer: A global perspective. American Institute for Cancer Research/World Cancer Research Fund, American Institute for Cancer Research, 1997. *Nutrition*, *15*, 523–526.

Goldin, B. R., & Gorbach, S. L. (1976). The relationship between diet and rat fecal bacterial enzymes implicated in colon cancer. *Journal of the National Cancer Institute*, *57*, 371–375.

Goldin, B. R., & Gorbach, S. L. (1984a). Alterations of the intestinal microflora by diet, oral antibiotics, and *Lactobacillus*: Decreased production of free amines from aromatic nitro compounds, azo dyes, and glucuronides. *Journal of the National Cancer Institute*, *73*, 689–695.

Goldin, B. R., & Gorbach, S. L. (1984b). The effect of milk and lactobacillus feeding on human intestinal bacterial enzyme activity. *The American Journal of Clinical Nutrition*, *39*, 756–761.

Goulet, O. (2015). Potential role of the intestinal microbiota in programming health and disease. *Nutrition Reviews*, *73*(Suppl 1), 32–40.

Han, K. J., Lee, N. K., Park, H., & Paik, H. D. (2015). Anticancer and anti-inflammatory activity of probiotic lactococcus lactis NK34. *Journal of Microbiology and Biotechnology*, *25*, 1697–1701.

Hassan, Z., Mustafa, S., Rahim, R. A., & Isa, N. M. (2016). Anti-breast cancer effects of live, heat-killed and cytoplasmic fractions of *Enterococcus faecalis* and *Staphylococcus hominis* isolated from human breast milk. In vitro cellular & developmental biology. *Animal*, *52*, 337–348.

Hassan, Z (2019). Anti-cancer and Biotherapeutic Potentials of Probiotic Bacteria. *Journal of Cancer Science & Therapy*, *11*.

Hill, M. J. (1975). The role of colon anaerobes in the metabolism of bile acids and steroids, and its relation to colon cancer. *Cancer*, *36*, 2387–2400.

Homey, B., Müller, A., & Zlotnik, A. (2002). Chemokines: Agents for the immunotherapy of cancer? Nature reviews. *Immunology*, *2*, 175–184.

Hotamisligil, G. S., & Erbay, E. (2008). Nutrient sensing and inflammation in metabolic diseases. Nature reviews. *Immunology*, *8*, 923–934.

Hsieh, P. S., Tsai, Y. C., Chen, Y. C., Teh, S. F., Ou, C. M., & King, V. A. (2012). Eradication of *Helicobacter pylori* infection by the probiotic strains *Lactobacillus johnsonii* MH-68 and *L. salivarius* ssp. salicinius AP-32. *Helicobacter*, *17*, 466–477.

Hussain, S. P., & Harris, C. C. (2007). Inflammation and cancer: An ancient link with novel potentials. *International Journal of Cancer*, *121*, 2373–2380.

Hwang, S. W., Kim, N., Kim, J. M., Huh, C. S., Ahn, Y. T., Park, S. H., et al. (2012). Probiotic suppression of the *H. pylori*-induced responses by conjugated linoleic acids in a gastric epithelial cell line. *Prostaglandins Leukot Essent Fatty Acids*, *86*, 225–231.

Ivanov, I. I., & Honda, K. (2012). Intestinal commensal microbes as immune modulators. *Cell Host & Microbe*, *12*, 496–508.

Iyer, C., Kosters, A., Sethi, G., Kunnumakkara, A. B., Aggarwal, B. B., & Versalovic, J. (2008). Probiotic *Lactobacillus reuteri* promotes TNF-induced apoptosis in human myeloid leukemia-derived cells by modulation of NF-kappaB and MAPK signalling. *Cellular Microbiology*, *10*, 1442–1452.

Jemal, A., Siegel, R., Ward, E., Hao, Y., Xu, J., Murray, T., et al. (2008). Cancer statistics, 2008. *CA: A Cancer Journal for Clinicians*, *58*, 71–96.

Jungersen, M., Wind, A., Johansen, E., Christensen, J. E., Stuer-Lauridsen, B., & Eskesen, D. (2014). The science behind the probiotic strain *Bifidobacterium animalis* subsp. lactis BB-12(®). *Microorganisms*, *2*, 92–110.

Kahouli, I., Tomaro-Duchesneau, C., & Prakash, S. (2013). Probiotics in colorectal cancer (CRC) with emphasis on mechanisms of action and current perspectives. *Journal of Medical Microbiology*, *62*, 1107–1123.

Kahouli, I., Malhotra, M., Alaoui-Jamali, M., & Prakash, S. (2015a). In-vitro characterization of the anti-cancer activity of the probiotic bacterium *Lactobacillus Fermentum* NCIMB 5221 and potential against colorectal cancer cells. *Journal of Cancer Science and Therapy*, *7*, 224–235.

Kahouli, I., Malhotra, M., Tomaro-Duchesneau, C., Rodes, L., Aloui-Jamali, M., & Prakash, S. (2015b). Identification of *Lactobacillus fermentum* strains with potential against colorectal cancer by characterizing short chain fatty acids production, anti-proliferative activity and survival in an intestinal fluid: In vitro analysis. *Bioanalysis & Biomedicine*, *7*, 104–115.

Kassayová, M., Bobrov, N., Strojný, L., Orendáš, P., Demečková, V., Jendželovský, R., et al. (2016). Anticancer and immunomodulatory effects of *Lactobacillus plantarum* LS/07. Inulin and melatonin in NMU-induced rat model of breast cancer. *Anticancer Research*, *36*, 2719–2728.

Kelly, DL, Lyon, DE, Yoon, SL, et al. (2016). The Microbiome and Cancer: Implications for Oncology Nursing Science. *Cancer Nurs*, *39*, E56–62.

Kim, M. H., Yoo, H. S., Chang, H. J., Hong, M. H., Kim, H. D., Chung, I. J., et al. (2005). Urokinase plasminogen activator receptor is upregulated by *Helicobacter pylori* in human gastric cancer AGS cells via ERK, JNK, and AP-1. *Biochemical and Biophysical Research Communications*, *333*, 874–880.

Kim, J. M., Kim, J. S., Kim, Y. J., Oh, Y. K., Kim, I. Y., Chee, Y. J., et al. (2008a). Conjugated linoleic acids produced by *Lactobacillus* dissociates IKK-gamma and Hsp90 complex in *Helicobacter pylori*-infected gastric epithelial cells. *Laboratory Investigation*, *88*, 541–552.

Kim, Y., Lee, D., Kim, D., Cho, J., Yang, J., Chung, M., et al. (2008b). Inhibition of proliferation in colon cancer cell lines and harmful enzyme activity of colon bacteria by *Bifidobacterium adolescentis* SPM0212. *Archives of Pharmacal Research*, *31*, 468–473.

Kim, Y., Oh, S., Yun, H. S., Oh, S., & Kim, S. H. (2010). Cell-bound exopolysaccharide from probiotic bacteria induces autophagic cell death of tumour cells. *Letters in Applied Microbiology*, *51*, 123–130.

Kim, J-E, Kim, J, Lee, K, et al. (2007). Cancer chemopreventive effects of lactic acid bacteria. *Journal of microbiology and biotechnology*, *17*, 1227–1235.

Kita, H. (2011). Eosinophils: Multifaceted biological properties and roles in health and disease. *Immunological Reviews*, *242*, 161–177.

Klaassen, C. D., & Reisman, S. A. (2010). Nrf2 the rescue: Effects of the antioxidative/electrophilic response on the liver. *Toxicology and Applied Pharmacology*, *244*, 57–65.

Kullisaar, T., Zilmer, M., Mikelsaar, M., Vihalemm, T., Annuk, H., Kairane, C., et al. (2002). Two antioxidative lactobacilli strains as promising probiotics. *International Journal of Food Microbiology*, *72*, 215–224.

Kumar, M., Kumar, A., Nagpal, R., Mohania, D., Behare, P., Verma, V., et al. (2010). Cancer-preventing attributes of probiotics: An update. *International Journal of Food Sciences and Nutrition*, *61*, 473–496.

Kumar, R. S., Kanmani, P., Yuvaraj, N., Paari, K. A., Pattukumar, V., Thirunavukkarasu, C., et al. (2012). *Lactobacillus plantarum* AS1 isolated from south Indian fermented food Kallappam suppress 1,2-dimethyl hydrazine (DMH)-induced colorectal cancer in male Wistar rats. *Applied Biochemistry and Biotechnology*, *166*, 620–631.

Kumar, K. S., Sastry, N., Polaki, H., & Mishra, V. (2015). Colon cancer prevention through probiotics: An overview. *Journal of Cancer Science and Therapy*, *7*, 081–092.

Kumar, KS, Sastry, N, Polaki, H, et al. (2015). Colon Cancer Prevention through Probiotics: An Overview. *Journal of Cancer Science & Therapy*, *7*, 1–12.

Kuugbee, E. D., Shang, X., Gamallat, Y., Bamba, D., Awadasseid, A., Suliman, M. A., et al. (2016). Structural change in microbiota by a probiotic cocktail enhances the gut barrier and reduces cancer via TLR2 signaling in a rat model of colon cancer. *Digestive Diseases and Sciences*, *61*, 2908–2920.

Lang, N. P., Butler, M. A., Massengill, J., Lawson, M., Stotts, R. C., Hauer-Jensen, M., et al. (1994). Rapid metabolic phenotypes for acetyltransferase and cytochrome P4501A2 and putative exposure to food-borne heterocyclic amines increase the risk for colorectal cancer or polyps. *Cancer Epidemiology, Biomarkers & Prevention*, *3*, 675–682.

Le Leu, R. K., Brown, I. L., Hu, Y., Bird, A. R., Jackson, M., Esterman, A., et al. (2005). A synbiotic combination of resistant starch and *Bifidobacterium lactis* facilitates apoptotic deletion of carcinogen-damaged cells in rat colon. *The Journal of Nutrition*, *135*, 996–1001.

Le Leu, R. K., Hu, Y., Brown, I. L., Woodman, R. J., & Young, G. P. (2010). Synbiotic intervention of *Bifidobacterium lactis* and resistant starch protects against colorectal cancer development in rats. *Carcinogenesis*, *31*, 246–251.

Lee, J. W., Shin, J. G., Kim, E. H., Kang, H. E., Yim, I. B., Kim, J. Y., et al. (2004). Immunomodulatory and antitumor effects in vivo by the cytoplasmic fraction of *Lactobacillus casei* and *Bifidobacterium longum*. *Journal of Veterinary Science*, *5*, 41–48.

Lee, N. K., Park, J. S., Park, E., & Paik, H. D. (2007). Adherence and anticarcinogenic effects of *Bacillus polyfermenticus* SCD in the large intestine. *Letters in Applied Microbiology*, *44*, 274–278.

Lee, H. A., Kim, H., Lee, K. W., & Park, K. Y. (2015a). Dead nano-sized *Lactobacillus plantarum* inhibits azoxymethane/dextran sulfate sodium-induced colon cancer in Balb/c mice. *Journal of Medicinal Food*, *18*, 1400–1405.

Lee, N., Son, S., Jeon, E. B., Jung, G. H., Lee, J., & Paik, H. (2015b). The prophylactic effect of probiotic *Bacillus polyfermenticus* KU3 against cancer cells. *Journal of Functional Foods*, *14*, 513–518.

Lee, JW, Shin, JG, Kim, EH, et al. (2004). Immunomodulatory and antitumor effects in vivo by the cytoplasmic fraction of Lactobacillus casei and Bifidobacterium longum. *Journal of veterinary science*, *5*, 41–48.

Lenoir, M., Del Carmen, S., Cortes-Perez, N. G., Lozano-Ojalvo, D., Muñoz-Provencio, D., Chain, F., et al. (2016). *Lactobacillus casei* BL23 regulates Treg and Th17 T-cell populations and reduces DMH-associated colorectal cancer. *Journal of Gastroenterology*, *51*, 862–873.

Li, Y., Schwabe, R. F., DeVries-Seimon, T., Yao, P. M., Gerbod-Giannone, M. C., Tall, A. R., et al. (2005). Free cholesterol-loaded macrophages are an abundant source of tumor necrosis factor-alpha and interleukin-6: Model of NF-kappaB- and map kinase-dependent inflammation in advanced atherosclerosis. *The Journal of Biological Chemistry*, *280*, 21763–21772.

Li, S., Huang, X. L., Sui, J. Z., Chen, S. Y., Xie, Y. T., Deng, Y., et al. (2014). Meta-analysis of randomized controlled trials on the efficacy of probiotics in *Helicobacter pylori* eradication therapy in children. *European Journal of Pediatrics*, *173*, 153–161.

Li, J., Sung, C. Y., Lee, N., Ni, Y., Pihlajamäki, J., Panagiotou, G., et al. (2016). Probiotics modulated gut microbiota suppresses hepatocellular carcinoma growth in mice. *Proceedings of the National Academy of Sciences of the United States of America*, *113*, E1306–E1315.

Lili, Z., Junyan, W., Hongfei, Z., Baoqing, Z., & Bolin, Z. (2018). Detoxification of cancerogenic compounds by lactic acid bacteria strains. *Critical Reviews in Food Science and Nutrition*, *58*, 2727–2742.

Lin, Y., Jiang, M., Chen, W., Zhao, T., & Wei, Y. (2019). Cancer and ER stress: Mutual crosstalk between autophagy, oxidative stress and inflammatory response. *Biomedicine & Pharmacotherapy*, *118*, 109249.

Linsalata, M., Cavallini, A., Messa, C., Orlando, A., Refolo, M. G., & Russo, F. (2010). *Lactobacillus rhamnosus* GG influences polyamine metabolism in HGC-27 gastric cancer cell line: A strategy toward nutritional approach to chemoprevention of gastric cance. *Current Pharmaceutical Design*, *16*, 847–853.

Liu, CF, & Pan, T-M (2010). In Vitro Effects of Lactic Acid Bacteria on Cancer Cell Viability and Antioxidant Activity. *Journal of Food and Drug Analysis*, *18*, 77–86.

Liu, C. F., & Pan, T. M. (2010). In vitro effects of lactic acid bacteria on cancer cell viability and antioxidant activity. *Journal of Food and Drug Analysis*, *18*, 77–86.

Louis, P., Hold, G. L., & Flint, H. J. (2014). The gut microbiota, bacterial metabolites and colorectal cancer. Nature reviews. *Microbiology*, *12*, 661–672.

Müller, J. M., Cahill, M. A., Rupec, R. A., Baeuerle, P. A., & Nordheim, A. (1997). Antioxidants as well as oxidants activate c-fos via Ras-dependent activation of extracellular-signal-regulated kinase 2 and Elk-1. *European Journal of Biochemistry*, *244*, 45–52.

Ma, E. L., Choi, Y. J., Choi, J., Pothoulakis, C., Rhee, S. H., & Im, E. (2010). The anticancer effect of probiotic *Bacillus polyfermenticus* on human colon cancer cells is mediated through ErbB2 and ErbB3 inhibition. *International Journal of Cancer*, *127*, 780–790.

Maccaferri, S., Klinder, A., Cacciatore, S., Chitarrari, R., Honda, H., Luchinat, C., et al. (2012). In vitro fermentation of potential prebiotic flours from natural sources: Impact on the human colonic microbiota and metabolome. *Molecular Nutrition & Food Research*, *56*, 1342–1352.

Maher, J., & Yamamoto, M. (2010). The rise of antioxidant signaling—The evolution and hormetic actions of Nrf2. *Toxicology and Applied Pharmacology*, *244*, 4–15.

Malaguarnera, G., Leggio, F., Vacante, M., Motta, M., Giordano, M., Bondi, A., et al. (2012). Probiotics in the gastrointestinal diseases of the elderly. *The Journal of Nutrition, Health & Aging*, *16*, 402–410.

Marotta, F., Naito, Y., Minelli, E., Tajiri, H., Bertuccelli, J., Wu, C. C., et al. (2003). Chemopreventive effect of a probiotic preparation on the development of preneoplastic and neoplastic colonic lesions: An experimental study. *Hepato-Gastroenterology*, *50*, 1914–1918.

Marteau, P., Pochart, P., Flourié, B., Pellier, P., Santos, L., Desjeux, J. F., et al. (1990). Effect of chronic ingestion of a fermented dairy product containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* on metabolic activities of the colonic flora in humans. *The American Journal of Clinical Nutrition*, *52*, 685–688.

Marteau, P, Pochart, P, Flourié, B, et al. (1990). Effect of chronic ingestion of a fermented dairy product containing Lactobacillus acidophilus and Bifidobacterium bifidum on metabolic activities of the colonic flora in humans. *The American journal of clinical nutrition*, *52*, 685–688.

Matsuzaki, T., Yokokura, T., & Mutai, M. (1988). Antitumor effect of intrapleural administration of *Lactobacillus casei* in mice. *Cancer Immunology, Immunotherapy*, *26*, 209–214.

Matsuzaki, T. (1998). Immunomodulation by treatment with *Lactobacillus casei* strain Shirota. *International Journal of Food Microbiology*, *41*, 133–140.

Matuskova, Z., Siller, M., Tunkova, A., Anzenbacherova, E., Zacharova, A., Tlaskalova-Hogenova, H., et al. (2011). Effects of *Lactobacillus casei* on the expression and the activity of cytochromes P450 and on the CYP mRNA level in the intestine and the liver of male rats. *Neuro Endocrinology Letters*, *32*(Suppl 1), 8–14.

Mazar, A. P. (2001). The urokinase plasminogen activator receptor (uPAR) as a target for the diagnosis and therapy of cancer. *Anti-Cancer Drugs*, *12*, 387–400.

Mcguire S (2016) World Cancer Report 2014. Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, WHO Press, 2015. Adv Nutr 7:418–419

McIntosh, G. H., Royle, P. J., & Playne, M. J. (1999). A probiotic strain of *L. acidophilus* reduces DMH-induced large intestinal tumors in male Sprague-Dawley rats. *Nutrition and Cancer*, *35*, 153–159.

McMahon, B. J., & Kwaan, H. C. (2015). Components of the plasminogen-plasmin system as biologic markers for cancer. *Advances in Experimental Medicine and Biology*, *867*, 145–156.

Mego, M, Holec, V, Drgona, L, et al. (2013). Probiotic bacteria in cancer patients undergoing chemotherapy and radiation therapy. *Complementary Therapies in Medicine*, *21*, 712–723.

Meyer-Rosberg, K., Scott, D. R., Rex, D., Melchers, K., & Sachs, G. (1996). The effect of environmental pH on the proton motive force of *Helicobacter pylori*. *Gastroenterology*, *111*, 886–900.

Mohania, D., Kansal, V. K., Kruzliak, P., & Kumari, A. (2014). Probiotic Dahi containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* modulates the formation of aberrant crypt foci, mucin-depleted foci, and cell proliferation on 1,2-dimethylhydrazine-induced colorectal carcinogenesis in Wistar rats. *Rejuvenation Research*, *17*, 325–333.

Moorehead, R. J., Hoper, M., & McKelvey, S. T. (1987). Assessment of ornithine decarboxylase activity in rectal mucosa as a marker for colorectal adenomas and carcinomas. *The British Journal of Surgery*, *74*, 364–365.

Nakanishi, S., Kataoka, K., Kuwahara, T., & Ohnishi, Y. (2003). Effects of high amylose maize starch and *Clostridium butyricum* on metabolism in colonic microbiota and formation of azoxymethane-induced aberrant crypt foci in the rat colon. *Microbiology and Immunology*, *47*, 951–958.

Nami, Y, Abdullah, N, Haghshenas, B, et al. (2014). A newly isolated probiotic Enterococcus faecalis strain from vagina microbiota enhances apoptosis of human cancer cells. *Journal of Applied Microbiology*, *117*, 498–508.

Noach, L. A., Bosma, N. B., Jansen, J., Hoek, F. J., van Deventer, S. J., & Tytgat, G. N. (1994). Mucosal tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-8 production in patients with *Helicobacter pylori* infection. *Scandinavian Journal of Gastroenterology*, *29*, 425–429.

Nouri, Z., Karami, F., Neyazi, N., Modarressi, M. H., Karimi, R., Khorramizadeh, M. R., et al. (2016). Dual anti-metastatic and anti-proliferative activity assessment of two probiotics on HeLa and HT-29 cell lines. *Cell Journal*, *18*, 127–134.

Nowak, A., Kuberski, S., & Libudzisz, Z. (2014). Probiotic lactic acid bacteria detoxify *N*-nitrosodimethylamine. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, *31*, 1678–1687.

Orlando, A., Refolo, M. G., Messa, C., Amati, L., Lavermicocca, P., Guerra, V., et al. (2012). Antiproliferative and proapoptotic effects of viable or heat-killed *Lactobacillus paracasei* IMPC2.1 and *Lactobacillus rhamnosus* GG in HGC-27 gastric and DLD-1 colon cell lines. *Nutrition and Cancer*, *64*, 1103–1111.

Ostaff, M. J., Stange, E. F., & Wehkamp, J. (2013). Antimicrobial peptides and gut microbiota in homeostasis and pathology. *EMBO Molecular Medicine*, *5*, 1465–1483.

Park, E., Jeon, G. I., Park, J. S., & Paik, H. D. (2007). A probiotic strain of *Bacillus polyfermenticus* reduces DMH induced precancerous lesions in F344 male rat. *Biological & Pharmaceutical Bulletin*, *30*, 569–574.

Parke, D. V., & Ioannides, C. (1990). Role of cytochromes P-450 in mouse liver tumor production. *Progress in Clinical and Biological Research*, *331*, 215–230.

Patel, B., Kumar, P., Banerjee, R., Basu, M., Pal, A., Samanta, M., et al. (2016). *Lactobacillus acidophilus* attenuates *Aeromonas hydrophila* induced cytotoxicity in catla thymus macrophages by modulating oxidative stress and inflammation. *Molecular Immunology*, *75*, 69–83.

Pourhoseingholi, M. A., Vahedi, M., & Baghestani, A. R. (2015). Burden of gastrointestinal cancer in Asia; an overview. *Gastroenterology and Hepatology from Bed to Bench*, *8*, 19–27.

Rao, D. N., & Ganesh, B. (1998). Estimate of cancer incidence in India in 1991. *Indian Journal of Cancer*, *35*, 10–18.

Rasouli, BS, Ghadimi-Darsajini, A, Nekouian, R, et al. (2017). In vitro activity of probiotic Lactobacillus reuteri against gastric cancer progression by downregulation of urokinase plasminogen activator/urokinase plasminogen activator receptor gene expression. *Journal of cancer research and therapeutics*, *13*, 246–251.

Rawla, P, & Barsouk, A (2019). Epidemiology of gastric cancer: global trends, risk factors and prevention. *Prz Gastroenterol*, *14*, 26–38.

Reddy, B. S., Mangat, S., Weisburger, J. H., & Wynder, E. L. (1977). Effect of high-risk diets for colon carcinogenesis on intestinal mucosal and bacterial beta-glucuronidase activity in F344 rats. *Cancer Research*, *37*, 3533–3536.

Richman, D. M., Tirumani, S. H., Hornick, J. L., Fuchs, C. S., Howard, S., Krajewski, K., et al. (2017). Beyond gastric adenocarcinoma: Multimodality assessment of common and uncommon gastric neoplasms. *Abdominal Radiology (New York)*, *42*, 124–140.

Ritchie, M. L., & Romanuk, T. N. (2012). A meta-analysis of probiotic efficacy for gastrointestinal diseases. *PLoS One*, *7*, e34938.

Roebuck, K. A. (1999). Oxidant stress regulation of IL-8 and ICAM-1 gene expression: Differential activation and binding of the transcription factors AP-1 and NF-kappaB (review). *International Journal of Molecular Medicine*, *4*, 223–230.

Sadeghi-Aliabadi, H, Mohammadi, F, Fazeli, H, et al. (2014). Effects of Lactobacillus plantarum A7 with probiotic potential on colon cancer and normal cells proliferation in comparison with a commercial strain. *Iran J Basic Med Sci*, *17*, 815–819.

Salminen, S., Bouley, C., Boutron-Ruault, M. C., Cummings, J. H., Franck, A., Gibson, G. R., et al. (1998). Functional food science and gastrointestinal physiology and function. *The British Journal of Nutrition*, *80*(Suppl 1), S147–S171.

Schoppmann, S. F., Birner, P., Stöckl, J., Kalt, R., Ullrich, R., Caucig, C., et al. (2002). Tumor-associated macrophages express lymphatic endothelial growth factors and are related to peritumoral lymphangiogenesis. *The American Journal of Pathology*, *161*, 947–956.

Sekine, K., Toida, T., Saito, M., Kuboyama, M., Kawashima, T., & Hashimoto, Y. (1985). A new morphologically characterized cell wall preparation (whole peptidoglycan) from *Bifidobacterium* infantis with a higher efficacy on the regression of an established tumor in mice. *Cancer Research*, *45*, 1300–1307.

Sheikhpour, E., Noorbakhsh, P., Foroughi, E., Farahnak, S., Nasiri, R., & Neamatzadeh, H. (2018). A survey on the role of interleukin-10 in breast cancer: A narrative. *Reports of Biochemistry & Molecular Biology*, *7*, 30–37.

Shin, R., Itoh, Y., Kataoka, M., Iino-Miura, S., Miura, R., Mizutani, T., et al. (2016). Anti-tumor activity of heat-killed *Lactobacillus plantarum* BF-LP284 on Meth-A tumor cells in BALB/c mice. *International Journal of Food Sciences and Nutrition*, *67*, 641–649.

Shiotani, A., & Graham, D. Y. (2002). Pathogenesis and therapy of gastric and duodenal ulcer disease. *The Medical Clinics of North America*, *86*, 1447–1466.

Singh, J., Rivenson, A., Tomita, M., Shimamura, S., Ishibashi, N., & Reddy, B. S. (1997). *Bifidobacterium longum*, a lactic acid-producing intestinal bacterium inhibits colon cancer and modulates the intermediate biomarkers of colon carcinogenesis. *Carcinogenesis*, *18*, 833–841.

Sivan, A., Corrales, L., Hubert, N., Williams, J. B., Aquino-Michaels, K., Earley, Z. M., et al. (2015). Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science*, *350*, 1084–1089.

Smits, H. H., Engering, A., van der Kleij, D., de Jong, E. C., Schipper, K., van Capel, T. M., et al. (2005). Selective probiotic bacteria induce IL-10-producing regulatory T cells in vitro by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. *The Journal of Allergy and Clinical Immunology*, *115*, 1260–1267.

Soltan Dallal, M. M., Yazdi, M. H., Holakuyee, M., Hassan, Z. M., Abolhassani, M., & Mahdavi, M. (2012). *Lactobacillus casei* ssp. casei induced Th1 cytokine profile and natural killer cells activity in invasive ductal carcinoma bearing mice. *Iranian Journal of Allergy, Asthma, and Immunology*, *11*, 183–189.

Soltan Dallal, M. M., Mojarrad, M., Baghbani, F., Raoofian, R., Mardaneh, J., & Salehipour, Z. (2015). Effects of probiotic *Lactobacillus acidophilus* and *Lactobacillus casei* on colorectal tumor cells activity (CaCo-2). *Archives of Iranian Medicine*, *18*, 167–172.

Soltan Dallal, MM, Mojarrad, M, Baghbani, F, et al. (2015). Effects of probiotic Lactobacillus acidophilus and Lactobacillus casei on colorectal tumor cells activity (CaCo-2). *Archives of Iranian medicine*, *18*, 167–172.

Srivastava, S. K., Yadav, U. C., Reddy, A. B., Saxena, A., Tammali, R., Shoeb, M., et al. (2011). Aldose reductase inhibition suppresses oxidative stress-induced inflammatory disorders. *Chemico-Biological Interactions*, *191*, 330–338.

Stein, K., Borowicki, A., Scharlau, D., Schettler, A., Scheu, K., Obst, U., et al. (2012). Effects of synbiotic fermentation products on primary chemoprevention in human colon cells. *The Journal of Nutritional Biochemistry*, *23*, 777–784.

Thirabunyanon, M., & Hongwittayakorn, P. (2013). Potential probiotic lactic acid bacteria of human origin induce antiproliferation of colon cancer cells via synergic actions in adhesion to cancer cells and short-chain fatty acid bioproduction. *Applied Biochemistry and Biotechnology*, *169*, 511–525.

Tiptiri-Kourpeti, A., Spyridopoulou, K., Santarmaki, V., Aindelis, G., Tompoulidou, E., Lamprianidou, E. E., et al. (2016). *Lactobacillus casei* exerts anti-proliferative effects accompanied by apoptotic cell death and up-regulation of TRAIL in colon carcinoma cells. *PLoS One*, *11*, e0147960.

Tiptiri-Kourpeti A, Spyridopoulou K, Santarmaki V et al. (2016) Lactobacillus casei Exerts Anti-Proliferative Effects Accompanied by Apoptotic Cell Death and Up-Regulation of TRAIL in Colon Carcinoma Cells. In: PloS one. p e0147960

Uccello, M., Malaguarnera, G., Basile, F., D'agata, V., Malaguarnera, M., Bertino, G., et al. (2012). Potential role of probiotics on colorectal cancer prevention. *BMC Surgery*, *12*(Suppl 1), S35.

Verfaillie, T., Garg, A. D., & Agostinis, P. (2013). Targeting ER stress induced apoptosis and inflammation in cancer. *Cancer Letters*, *332*, 249–264.

Virchow, R. (1989). Cellular pathology. As based upon physiological and pathological histology. Lecture XVI—Atheromatous affection of arteries. 1858. *Nutrition Reviews*, *47*, 23–25.

Walia, S., Kamal, R., Kanwar, S. S., & Dhawan, D. K. (2015). Cyclooxygenase as a target in chemoprevention by probiotics during 1,2-dimethylhydrazine induced colon carcinogenesis in rats. *Nutrition and Cancer*, *67*, 603–611.

Wang, A. N., Yi, X. W., Yu, H. F., Dong, B., & Qiao, S. Y. (2009). Free radical scavenging activity of *Lactobacillus fermentum* in vitro and its antioxidative effect on growing-finishing pigs. *Journal of Applied Microbiology*, *107*, 1140–1148.

Wang, L. X., Liu, K., Gao, D. W., & Hao, J. K. (2013). Protective effects of two *Lactobacillus plantarum* strains in hyperlipidemic mice. *World Journal of Gastroenterology*, *19*, 3150–3156.

Wang, Y., Wu, Y., Wang, Y., Fu, A., Gong, L., Li, W., et al. (2017). *Bacillus amyloliquefaciens* SC06 alleviates the oxidative stress of IPEC-1 via modulating Nrf2/Keap1 signaling pathway and decreasing ROS production. *Applied Microbiology and Biotechnology*, *101*, 3015–3026.

Wang, M, Zhao, J, Zhang, L, et al. (2017). Role of tumor microenvironment in tumorigenesis. *J Cancer*, *8*, 761–773.

Yamaoka, Y., & Graham, D. Y. (2014). *Helicobacter pylori* virulence and cancer pathogenesis. *Future Oncology (London, England)*, *10*, 1487–1500.

Yamazaki, K., Tsunoda, A., Sibusawa, M., Tsunoda, Y., Kusano, M., Fukuchi, K., et al. (2000). The effect of an oral administration of *Lactobacillus casei* strain Shirota on azoxymethane-induced colonic aberrant crypt foci and colon cancer in the rat. *Oncology Reports*, *7*, 977–982.

Yang, Y. J., & Sheu, B. S. (2012). Probiotics-containing yogurts suppress *Helicobacter pylori* load and modify immune response and intestinal microbiota in the *Helicobacter pylori*-infected children. *Helicobacter*, *17*, 297–304.

Yang, Y. J., Chuang, C. C., Yang, H. B., Lu, C. C., & Sheu, B. S. (2012). *Lactobacillus acidophilus* ameliorates *H. pylori*-induced gastric inflammation by inactivating the Smad7 and NFκB pathways. *BMC Microbiology*, *12*, 38.

Yasui, H., Shida, K., Matsuzaki, T., & Yokokura, T. (1999). Immunomodulatory function of lactic acid bacteria. *Antonie van Leeuwenhoek*, *76*, 383–389.

Zaki, M., Coudron, P. E., McCuen, R. W., Harrington, L., Chu, S., & Schubert, M. L. (2013). *H. pylori* acutely inhibits gastric secretion by activating CGRP sensory neurons coupled to stimulation of somatostatin and inhibition of histamine secretion. *American Journal of Physiology—Gastrointestinal and Liver Physiology*, *304*, G715–G722.

Zangar, R. C., Davydov, D. R., & Verma, S. (2004). Mechanisms that regulate production of reactive oxygen species by cytochrome P450. *Toxicology and Applied Pharmacology*, *199*, 316–331.

Zhang, H. L., Yu, L. X., Yang, W., Tang, L., Lin, Y., Wu, H., et al. (2012). Profound impact of gut homeostasis on chemically-induced pro-tumorigenic inflammation and hepatocarcinogenesis in rats. *Journal of Hepatology*, *57*, 803–812.

Zhang, M., Wang, F., Jiang, L., Liu, R., Zhang, L., Lei, X., et al. (2013). *Lactobacillus salivarius* REN inhibits rat oral cancer induced by 4-nitroquinoline 1-oxide. *Cancer Prevention Research*, *6*, 686–694.

Zhang, D. D. (2006). Mechanistic studies of the Nrf2-Keap1 signaling pathway. *Drug Metabolism Reviews*, *38*, 769–789.

Zhang, HL, Yu, LX, Yang, W, et al. (2012). Profound impact of gut homeostasis on chemically-induced pro-tumorigenic inflammation and hepatocarcinogenesis in rats. *Journal of hepatology*, *57*, 803–812.

Zhong, L., Zhang, X., & Covasa, M. (2014). Emerging roles of lactic acid bacteria in protection against colorectal cancer. *World Journal of Gastroenterology*, *20*, 7878–7886.

Zhu, XY, & Liu, F (2017). Probiotics as an adjuvant treatment in Helicobacter pylori eradication therapy. *Journal of Digestive Diseases*, *18*, 195–202.

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Chapter 13

Psychobiotics: A Newer Approach Toward the Treatment of Neurodevelopmental Disorders

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1 Introduction

The skin and mucosal surfaces of vertebrates contain a wide assortment of microorganisms (all things considered named microbiota) that incorporates microscopic organisms, growths, parasites, and infections. The human gut harbors some of the most complex and abundant ecosystems composed of up to 1013–1014 microorganisms, which is between 1 and 10 occasions more than the quantity of eukaryotic cells in the body (Leung & Thuret, 2015; Qin, 2010a). The aggregate of grown-up human gut microbiota is made of a limit of 500–1000 bacterial species (Leung & Thuret, 2015; Palmer, Bik, DiGiulio, Relman, & Brown, 2007; Jasarevic, Howerton, Howard, & Bale, 2015). Many long stretches of coadvancement have prompted a common beneficial interaction between the host and gut microbiome. In fact, the gut is wealthy in particles that can be utilized as supplements by the microorganisms, preferring microbiota colonization (Leung & Thuret, 2015). Gut colonization starts during childbirth and is set up in the first 3 years of life. The underlying cooperation between gut microbiota and the host is fundamental for the development of the sensory system, the invulnerable framework, and for the formative guideline of intestinal physiology (Leung & Thuret, 2015; Bercik, Collins, & Verdu, 2012; Kamada, Seo, Chen, & Nunez, 2013). At this stage, gut microbiota is likewise ready to adjust the procedure of angiogenesis (Collins & Bercik, 2009). Besides, microorganisms likewise show against microbial exercises in way keeping up a steady gut biological system. Modifications during the time spent microbial colonization of the human gut in early life have been appeared to influence the danger of malady (Moustafa et al., 2018). Further down the road, microbial colonization of the digestive system has a significant sway on the host neurophysiology, conduct and capacity of the sensory system (Fung, Olson, & Hsiao, 2017). Given the immunomodulatory properties of gut microbiota, it has been demonstrated that different resistant pathways, inside and outside the focal apprehensive system, Central Nervous System (CNS), are engaged with significant instrument like microbial intercession of cerebrum capacities and conduct. It has been found that neuro-immunomodulation by the microbiota can add to etiopathogenesis or to show significant signs and manifestations in neurodegenerative and social issue, for example, chemical imbalance range issue, Autism Spectrum Disorder (ASD), uneasiness, gloom, Alzheimer's sickness (AD), and Parkinson's infection (PD) (Fung et al., 2017). Moreover, different microscopic organisms, which are usually present in a huge decent variety of food items, travel through our gut each day, communicating with the food

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00013-7
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items themselves, the host microbiota, and our own cells in either a sound or an obsessive setting. Huge numbers of the last microorganisms are known as probiotics. Probiotics are defined as "live microorganisms which when regulated in sufficient sums present a well-being benefit on the host" (Hill et al., 2014). This idea depends on the perceptions made by Élie Metchnikoff in 1907 in which the normal utilization of lactic corrosive microscopic organisms, Lactic acid bacilli (LAB) in aged dairy items, for example, yogurt was related with improved well-being and life span in numerous people.

In relationship with probiotics, the idea of prebiotic was first presented by Gibson and Roberfroid in 1995 as a "nondigestible food fixing that beneficially affects the host by specifically invigorating the development as well as movement of one or a set number of microbes effectively occupant in the colon." This definition infers their capacity to oppose to have absorption and their one of kind actions on microbiota intestinal. In any case, the advancement in prebiotic study has constrained the development of this definition, as they can likewise be straightforwardly controlled to different destinations of the body that are additionally colonized by commensal microorganisms, for example, skin or vagina. As of late, the International Scientific Association for Probiotics and Prebiotics (ISAPP) in an agreement board proposed another definition of prebiotics as "a substrate that is specifically used by having microorganisms giving a well-being benefit," taking into account that dietary ones must not be debased by having catalysts. Substrates that fit with this definition incorporate oligosaccharides (OSs), polyunsaturated fat, conjugated linoleic corrosive, plant polyphenols, and certain fermentable fibers. Among prebiotics feature, fructo-oligosaccharides (FOSs), inulin, galacto oligosaccharides (GOSs), mannan oligosaccharides (MOS), xylo OSs, and human milk OSs, being FOS and GOS the most concentrated just as the traditionally acknowledged ones. Prebiotics have been demonstrated to exert beneficial effects on the gastrointestinal tract, resistant framework, bones, lipid and sugar digestion, and on emotional well-being.

Burdensome issues like major depressive disorder (MDD), temperament issue, and stress are mind boggling and multifactorial issues that include checked incapacities in worldwide working, anorexia, and serious clinical comorbidities. MDD influences about 20% of the populace eventually, during the lifetime of an individual (Yamagishi et al., 2015). With a rising group of information on human microbiomes and its relationship with psychological wellness, it is turning out to be progressively evident that there are some missing connections between the inhabitant organisms and different parts of physiology. Reports from creature considered recommend collaborations of gut microbiota with the enteric sensory system as well as with the focal sensory system by means of neural, neuroendocrine, neuroimmune, and humoral connections. From ongoing confirmations, researchers are recommending that the neuroimmune framework may affect the creation of the microbiota. Be that as it may, the advanced way of life factors (diet, stress, and physical movement), antibacterial items, ecological components (contaminants in food), and antimicrobials both in food and as medication may drain such organisms that are beneficial for physical, mental, and passionate well-being. Insufficiency of such beneficial microorganisms may prompt well-being outcomes. Confirmations from creature and human examinations have demonstrated that the organization of beneficial microorganisms can decrease gloom, tension, push and improve cognizance, recommending opportunities for another class of probiotics known as "psychobiotics." Psychobiotics are a gathering of probiotics, fit for creating and conveying neuroactive substances, for example, gamma-aminobutyric corrosive, and serotonin (the "glad" compound) that demonstrate over the cerebrum gut pivot. Especially, it can likewise decrease the degrees of cortisol (the "stress" hormone) and increment levels of oxytocin (the "nestle" hormone) (Mayer, Knight, Mazmanian, Cryan, & Tillisch, 2011; Selhub, Logan, & Bested, 2014).

2 Gut microbiota as psychobiotics

Psychobiotics are the latest of those specialists with guessed psychotropic properties. Dinan et al. defined psychobiotics as those living creatures, upon sufficient ingestion deliver a well-being benefit to patients with mental diseases. Hence, the expression "psychobiotics" assigns this novel class of probiotics, which has more extensive applications in mental medication (Messaoudi, Lalonde, & Violle, 2011a). Various commensal gut organisms, customarily known as probiotics (*Lactobacillus* spp., *Bifidobacterium* spp., and *Saccharomyces* spp.), apply a few beneficial well-being impacts and are used to treat intestinal dysbiosis. Table 13.1 presents instances of gut organisms with psychotropic properties. Interestingly, a portion of the intestinal microorganisms, for example, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Escherichia*, *Bacillus*, *Saccharomyces*, *Candida*, *Streptococcus*, and *Enterococcus* can create synapses, including serotonin, norepinephrine, and gamma-aminobutyric corrosive. They likewise can balance the articulation of neurochemical receptors like endocannabinoid receptors and follow up on the proposed mind gut pivot bringing about psychotropic impacts (upper and anxiolytic) (Ek, 2001; Erny, 2015; Leung & Thuret, 2015). It has additionally been accounted for that an oral administration of a mix of probiotics, *Lactobacillus helveticus* R0052 and *B. longum* R0175 (Probio'Stick) for a time of 1 month, improves gloom, outrage, and nervousness and brings down the degree of the pressure hormone cortisol (Messoudi et al., 2011a) (Table 13.2). A little fake treatment-controlled examination, including Functional magnetic resonance imaging (FMRI), has likewise exhibited that a 1-month utilization of a matured food

TABLE 13.1 Gut microbiota with psychotrophic properties.

Lactobacillus spp.	*L. acidophilus*
	L. casei
	L. rhamnosus
	L. helveticus
	L. plantarum
	L pentosus
	L casei Shirota
	L. hilgardii
Bifidobacterium spp.	*B. infantis*
	B. longum
	B. bifidum
	B. lactis
	B. breve
Other spp.	*Bacillus*
	Candida
	Enterococcus
	Escherichia
	Streptococcus thermophilus
	Saccharomyces spp.

containing *Bifidobacterium animalis* subsp. lactis, *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Lactococcus lactis* subsp. lactis can influence mind action when contrasted with standard (Tillisch, Labus, & Kilpatrick, 2013a).

3 Prebiotics for psychobiotics

The connection between matured dairy items and the development of beneficial intestinal microorganisms is very much considered. In any case, ongoing reports have shown that (nondairy) matured nourishments and herbs can likewise have a positive influence on the intestinal microbiota. These are similarly significant, as there might be an influence on longer term gut-mind correspondence.

Proof likewise proposes that the well-being advancing objective of flavonoids coordinates toward the human gut bacterial metagenomes, whose benefits have a transformative root. Utilitarian investigation utilizing bunches of orthologous gatherings of microscopic organism's target proteins recommends that flavonoids manage the digestion of gut microbiota. Trial research has demonstrated that when basic dietary polyphenols are exposed to maturation, the recently framed biotransformation phytochemicals are progressively equipped for causing a beneficial move in microbial development incitement (Selhub et al., 2014). For instance, isomalto OSs are found in customary nourishments (nectar, purpose, miso, and soy sauce), matured cabbage (kimchi), aged fish oil, and fiber-rich segments of conventional eating regimens (soy germ, wheat germ, rice grain, or breads). They have appeared in creatures and people to have a beneficial impact on advancing the development of *Bifidobacteria* and *Lactobacillus* sp. (Selhub et al., 2014; Messaoudi et al., 2011b).

4 Psychophysiological effects of psychobiotics

Much psychobiotic research depends on rat models, which use rat pressure enlistments and rat social tests to survey inspiration, uneasiness, and wretchedness. Psychobiotics applied to rat models of sickness, contamination, and neurodegeneration additionally give early clinical understanding into human illnesses (Table 13.1). Human examinations speak to an ongoing pattern. The psychophysiological impacts of psychobiotics fall into the accompanying three classes: (1) Psychological consequences for passionate and intellectual procedures. (2) Systemic consequences for the HPA pivot and the glucocorticoid stress reaction, and irritation that is regularly described by abnormal cytokine fixations. Genius fiery cytokines share a solid and all around considered positive relationship with mental conditions, for example, sadness (Yamagishi et al., 2015). For instance, infusion

TABLE 13.2 Psychobiotics used in neurological conditions.

Neurological condition	Gut microbes	Psychobiotic strains
Anxiety	*Lactobacillus* spp.	*L. fermentum NS9*
		L. casei Shirota
		L. rhamnosus JB-1
		L. helveticus ROO52
	Bifidobacterium spp.	*B. breve 1205*
		B. infantis
		B. longum 1714
		B. longum NCC3001
		B. longum R0175
Depression	*Lactobacillus* spp.	*L. acidophilus*
		L. acidophilus W37
		L. brevis W63
		L. casei
		L. casei Shirota
		L. casei W56
		L. gasseri OLL2809
		L. helveticus NS8
	Lactococcus spp.	*L. lactis W19*
		L. lactis W58
	Bifidobacterium spp.	*B. infantis*
		B. bifidum
		B. bifidum W23
		B. lactis W52
		B. longum R0175
Stress	*Lactobacillus* spp.	*L. casei Shirota*
		L. helveticus
		L. helveticus R0052
		L. plantarum PS128
		L. rhamnosus
	Bifidobacterium spp.	*B. infantis*
		B. longum R0175

of interferon-α, an ace fiery cytokine, has been appeared to actuate discouragement, which can be eased through upper activity (Kessler et al., 2005; Mayer et al., 2011). (3) Neural impacts on synapses and proteins. Important synapses incorporate Gamma-Aminobutyric Acid (GABA) and glutamate, which control neural excitation–hindrance balance. Proteins incorporate mind determined Brain Derived Neurotrophic Factor (BDNF), which assumes an essential job in learning and memory forms, including spatial learning, eradication of adapted dread, and article acknowledgment (Selhub et al., 2014; Messaoudi et al., 2011a). BDNF is decreased in nervousness and misery, a decrease that is reversible through upper activity (Tillisch et al., 2013a).

5 Microbes–brain signaling

The instruments through which psychobiotics apply their belongings still cannot seem to be plainly characterized and remain inadequately comprehended. Despite the fact that there are a few investigations that give unthinking bits of knowledge to people, most of exploration depends on rat models. A significant advance in creating information on the systems lies in examining how the microbiome and the cerebrum speak with each other (see Fig. 13.1).

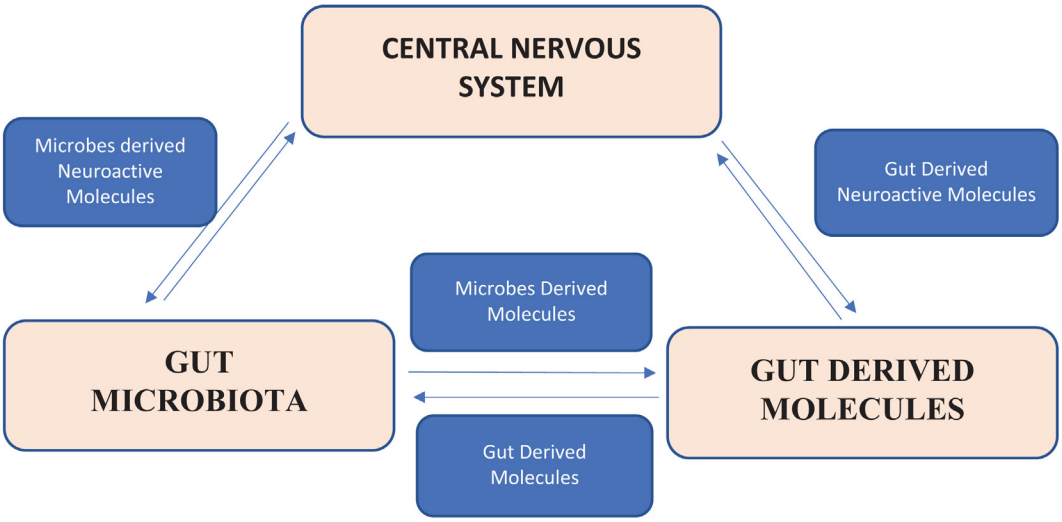


FIGURE 13.1 Microbes Brain Signalling

6 Mind-enteric nervous system interaction

Gut microscopic organisms control electrophysiological limits in enteric sensory system neurons. For instance, myenteric neurons presented to *B. longum* NCC3001-aged substances indicated a decreased age of activity possibilities when they were electrically animated (Dowlati, 2010). Essentially, colonic AH neurons (the boss tactile neurons in the colon) rewarded with *Lactobacillus rhamnosus* demonstrated expanded volatility, an impact that rose up out of hindrance of calcium-controlled potassium entryways (Udina, 2012). Other work demonstrated that neurons from the dorsal root ganglion in the colon did not show hyperexcitability in light of toxic incitement in the event that they had been treated with *L. rhamnosus*. Myenteric neurons are likewise in nearness to the gut lumen that would encourage their contact with the microbiome. In sans germ mice, these neurons show lower levels of sensitivity contrasted with their typically colonized partners (McNutt, 2012). One investigation discovered a proof of intestinal neural variations from the norm in the jejunum and ileum of without germ mice in contrast with controls (Lu, 2008), with sans germ mice indicating diminished nerve thickness, less nerves per ganglion, and a more noteworthy number of myenteric nitrergic neurons. Ongoing proof likewise demonstrates that the microbiome influences particle transport constrained by cyclic adenosine monophosphate (Heldt, 2007).

In general, these outcomes give a striking proof of direct, microbes prompted adjustment of the enteric sensory system. Additionally, the impact of the microbiome on the enteric sensory system reaches out past neurons, with ongoing discoveries showing that gut microscopic organisms likewise assume a vital job in the turn of events and homeostasis of glial populaces in the gut (Martinowich & Lu, 2008).

Gut microscopic organisms additionally produce a scope of synapses through the digestion of toxic strands. These incorporate dopamine and noradrenalin by individuals from the *Bacillus* family, GABA by the *Bifidobacteria* family, serotonin by the *Enterococcus* and *Streptococcus* families, noradrenalin and serotonin by the *Escherichia* family, and GABA and acetylcholine by the *Lactobacilli* family (Barrett, Ross, O'Toole, Fitzgerald, & Stanton, 2012; Dinan, 2015; Lyte, 2011). In spite of the fact that there is no immediate proof starting at yet, all things considered, these synapses tweak synaptic action in the proximal neurons of the enteric sensory system and are a significant road for future exploration.

7 Vagal signaling

The vagus nerve plays a basic and wide-going job in planning parasympathetic action, including guideline of pulse and gut motility. It has a bounty of tangible filaments and can pass on rich data on organ work all through the body to the cerebrum (Thayer & Sternberg, 2009). Vagal movement is touchy to sustenance, exercise, and stress (De Hann et al., 2015; Mezzacappa, 2001; Spalding, 2000).

Invigorating the vagus nerve applies mitigating impacts, and is utilized remedially for hardheaded wretchedness, torment, and epilepsy (Kirchner, 2000; Morris & Mueller, 1999; Sackeim, 2001; Rush, 2005). There is an additional proof of

the two antidepressants and anxiolytics applying vagal impacts (Adinoff, 1992; Salam, 2004; Smith, 2014), recommending that vagal balance might be a typical pathway for the impacts of antidepressants, anxiolytics, and psychobiotics.

A few creatures considered have discovered that the vagus nerve intercedes the connection among psychobiotics and their psychophysiological impacts, as cutting off the vagus nerve (vagotomy) nullifies reactions to psychobiotic organization (De Lartigue, 2011). Nonetheless, one examination has discovered that the ingestion of antimicrobials expanded characteristic relative plenitude of *Lactobacilli* in intrinsically on edge male BALB/c mice, a change that was joined by expanded exploratory conduct and BDNF articulation. Urgently, be that as it may, vagotomy did not dispense with these neural or social advantages (Bercik, 2011). Along these lines, vagal flagging might be all things considered a halfway middle person of bacterial impacts.

8 Short-chain fatty acids, gut hormones, and bacteria-derived blood metabolites

The human gut is unequipped for processing macronutrients, for example, plant polysaccharides. While these every now and again show up in the eating regimen, the human genome does not code the essential chemicals for their assimilation, which are provided by the microbiome (Qin et al., 2010b). The metabolization of these filaments delivers short-chain unsaturated fats (SCFAs), including acetic acid derivation, butyrate, lactate, and propionate (Horn & Klein, 2013; Moretti, 2011). SCFAs enter the circulatory framework through the digestive organ (Overduin, 2013), where the more prominent extent is coordinated into the liver and muscle. In spite of the fact that it is indistinct how much the little part of SCFAs crossing into the focal sensory system balances neurotransmission, there is some proof for their psychotropic properties at pharmacological focuses. For example, foundational sodium butyrate infusions (200 mg/kg body weight) in rodents produce upper impacts, and increment in focal serotonin neurotransmission and BDNF articulation (Sun, 2016). Here, the activity of butyrate as an epigenetic modifier (Stilling, 2014) is almost certain contrasted with activity as an agonist at a Free Unsaturated fatty acid receptor (FFAR), given that there are not many FFARs in the cerebrum (Erny, 2015). In any case, it ought to be noticed that the Short Chain Fatty Acids (SCFAs) show pleiotropy (autonomous impacts created by a solitary quality) and furthermore animate the HPA hub (Gagliano, 2014) or affect the mucosal insusceptible framework (Alenghat & Artis, 2014), which may in a roundabout way influence focal neurotransmission. An ongoing rat examination (Perry, 2016) has additionally discovered that the SCFA acetic acid derivation assumes a causal job in weight. Acetic acid derivation produced by the gut microscopic organisms because of high-fat eating regimens triggers parasympathetic movement and advances increments in ghrelin, glucose-animated insulin, and further nourishment admission, making a positive input circle that improves the probability of stoutness.

SCFAs additionally impact the emission of satiety peptides, including cholecystokinin, peptide tyrosine (PYY), and glucagon-like peptide-1 (GLP-1), from gut mucosal enteroendocrine cells that express FFARs (Nøhr, 2013). For example, propionic corrosive intercedes the arrival of GLP-1 and Peptide Tyrosine Tyrosine (PYY) through actuation of FFAR2 (Psichas, 2015). Reliable with the idea that SCFAs are delivered from the bacterial digestion of dietary polysaccharides, prebiotic supplementation expands the creation of intestinal SCFAs, which regulate enteroendocrine cells and their discharge of PYY and GLP-1 (Cani, 2005). It is in this way sensible that the satiety hormones may assume an increasingly huge job in the focal impacts of prebiotics contrasted with probiotics. Besides, circling PYY and GLP-1 have mind penetrant properties, and their organization to rodents affect synapses and conduct (Dockray, 2014; Anderberg, 2014; Stadlbauer, 2015).

The microbiome has additionally been appeared to have a significant job in creating metabolites that enter flow and apply a scope of results outside the gut (Morris, 2016). A key report that contrasted sans germ mice with regularly colonized mice discovered striking impacts of the microbiome on the decent variety and amount of blood metabolites (Wikoff, 2015). For example, without germ mice had 40% more noteworthy plasma tryptophan fixations than typical mice, yet the ordinary mice had 2.8 occasions more prominent plasma serotonin levels than the sans germ mice. This proposes that gut microorganisms urgently influence the digestion of tryptophan into serotonin in Enterochromaffin cells (serotonin-discharging cells inserted in the luminal epithelium). Despite the fact that the particular system through which microscopic organisms may control serotonin creation in Enterochromaffin cells was obscure around then, an ongoing report has ascribed this job to indigenous spore-framing microbes in the gut. There were correspondingly emotional contrasts in other tryptophan metabolites, particularly those containing indole, for example, the cancer prevention agent indole-3-propionic corrosive (IPA) and indoxyl sulfate, which were undetected in the sans germ mice and whose creation was hence deciphered as being completely intervened by gut microbes.

We guess that these metabolites are delicate to psychobiotic activity. In any case, the connections between the microbiome, microorganisms inferred metabolites, and the focal sensory system, just as the job of psychobiotics in adjusting this system, remain for all intents and purposes unexplored.

9 Microbes immune interactions

A key capacity of the insusceptible framework is to identify and dispense with pathogens. Each microorganism has an organism related subatomic example Microbe Associated Molecular Pattern (MAMP, recently alluded to as pathogen-related subatomic examples) (Mackey & McFall, 2006). A scope of tiny components may go about as MAMPs, including microbial nucleic acids, subatomic cell divider segments (e.g., lipopolysaccharides), or bacterial flagella. Gut microorganisms can speak with the enteric sensory system and the inborn insusceptible framework through communications between the MAMPs and example acknowledgment receptors inserted along the lumen. The group of example acknowledgment receptors incorporates Toll-like receptors (TLRs), C-type lectins, and inflammasomes. These receptors can recognize the nature and possible impacts of different organisms by means of the MAMPs and, at a wide level, transmit data about the microbial condition to the host, empowering explicit immunological reactions.

The MAMPs of advantageous microbes, by activating example acknowledgment receptors, may encourage the emission of mitigating cytokines, for example, interleukins (Chu & Mazmanian, 2013). While thorough robotic portrayals of the connection between MAMPs, design acknowledgment receptors, and decreases in aggravation are deficient with regards to, one interesting speculation is that advantageous microscopic organisms may fill in as physical hindrances that square pathogenic MAMPs (e.g., lipopolysaccharides) from initiating host design acknowledgment receptors, for example, TLR2 and TLR4 by authoritative to them rather, along these lines forestalling ace provocative reactions (Zhou, 2015).

Prebiotics may act in a comparative limit, as there is a proof of direct connection among OSs and the epithelium, autonomous of gut microbes, with significant decreases in professional incendiary cytokines (Bode, 2004; Eiwegger, 2010). Prebiotics may forestall pathogenic MAMPs from getting to design acknowledgment receptors, either by going about as physical obstructions to diminish the rate of MAMP official, or by legitimately authoritative to the receptor themselves. In this manner, prebiotics need not apply the entirety of their gainful impacts only by developing commensal microscopic organisms.

One component for psychobiotic impacts is the relief of second-rate irritation, commonly saw as decreases in flowing master provocative cytokine focuses. Professional provocative cytokines are additionally fit for expanding the penetrability of the blood–cerebrum hindrance (McCusker & Kelley, 2013), allowing access to expected pathogenic substances. Cytokines modify groupings of a few synapses that direct correspondence in the mind, including serotonin, dopamine, and glutamate (Miller, 2013). Cytokines can likewise enter the cerebrum through dynamic take-up, invigorating emission of ace provocative substances, for example, prostaglandins (Sun, 2016) hastening further aggravation. There is additionally developing proof of a lymphatic seepage framework supporting the mind (Louveau, 2015), which we guess may permit cytokines to cooperate with neural tissue.

An equal system fundamental psychobiotic-incited decrease in irritation is the expansion of calming cytokines, for example, interleukin-10. For instance, in people, *B. infantis* 35624 and *Lactobacillus* GG 38 is the strain (Pessi, 2000) have been appeared to improve centralizations of interleukin-10. By decreasing the all-out amount of genius incendiary cytokines, either legitimately or by expanding mitigating cytokines, psychobiotics might be lessening the likelihood of cytokines accessing the focal sensory system and may likewise be reestablishing irritation incited penetrability of the blood–mind boundary. Cytokine collaborations at the blood–mind hindrance are exceptionally unpredictable, and progressively point-by-point conversations of those systems are past the extent of this survey. The peruser is alluded to existing examination around there (Banks & Erickson, 2010; de Vries, 1996; Ek, 2001).

A parasitic disease study (Bercik, 2010) yielded a significant robotic knowledge with respect to cytokine jobs in microbiome–mind flagging. Solid male AKR mice were tainted with the *Trichuris muris* parasite, following which they were treated with *B. longum* NCC3001, *L. rhamnosus* NCC4007, or vehicle. Contamination expanded on edge conduct and diminished hippocampal BDNF mRNA levels. *B. longum* NCC3001 (yet not *L. rhamnosus* NCC4007) decreased on edge conduct and standardized BDNF mRNA focuses. Be that as it may, these progressions happened without prebiotic-prompted decreases in any master fiery cytokines. This might be deciphered as proof that psychobiotic impacts additionally happen through components other than cytokine decrease.

Professional fiery cytokines are additionally known to bargain the uprightness of the gut obstruction (Wang, 2006; Donato, 2010). For instance, *L. rhamnosus* GG improves gut boundary brokenness by hindering the flagging capability of star provocative cytokines, for example, tumor putrefaction factor-α (Donato, 2010).

The microbiome additionally assumes a significant job in more extensive immunological capacities. For instance, the nearness of microbes, for example, *B. infantis* 35624 and *Lactobacillus salivarius* UCC118 in the gut, has been appeared to influence immunogenic reactions toward pathogenic substances, for example, *Salmonella typhimurium* (O'Hara, 2006). The microbiome likewise adds to the advancement of the resistant framework. For instance, the statement of colonic effector star fiery qualities that are touchy to the activity of interleukin-10 is microscopic organisms subordinate (Redhu, 2016). Exogenous microbes can likewise trigger the improvement of immunogenic reactions under specific conditions. For

example, the presentation of Helicobacter hepaticus within the sight of hereditarily lacking interleukin-10 flagging frameworks prompted an expansion in genius provocative minor zone B cells (in the class of white platelets, communicating antibodies) of the spleen (Ray, 2015). The microbiome likewise controls the advancement of proper immunosuppression in light of dietary antigens through the creation of immunosuppressive administrative T-cells (Kim, 2016). These cells forestall full immunogenic responses to typical wholesome information, though without germ mice do not have this immunosuppressive action and demonstrate overstated resistant reactions to dietary antigens. These microorganisms' invulnerable communications delineate limit conditions for psychobiotics. For example, certain hereditary variations from the norm that change the invulnerable framework may bring about startling psychobiotic impacts.

10 Neuropsychological disorders

It has for quite some time been comprehended that solid microbiota assume a basic job in human turn of events, especially in territories, for example, safe turn of events and metabolism. Although in its beginning times, the rising field of human microbiome research has demonstrated that gut microbiota may likewise assume a significant job in affecting mental health, conduct, and disposition in humans (Tillisch et al., 2013b). In an ongoing report, sound female volunteers expended a matured milk item with a blend of probiotics, including *B. animalis* subsp. lactis, *S. thermophilus*, *L. bulgaricus*, and *L. lactis* subsp. lactis, for about a month. Results demonstrated that probiotic utilization impacted cerebrum action in passionate focuses in solid individuals (Tillisch et al., 2013a). The connection between stress, microbiota, and conduct is a significant zone of examination in the field (Dinan & Cryan, 2012). One investigation indicated improved disposition in sound volunteers following 3-week utilization of a probiotic-containing milk drink that contained *L. casei* Shirota. More as of late, a 30-day utilization of a probiotic blend containing *L. helveticus* and *B. longum* indicated helpful impacts on uneasiness and burdensome measures and demonstrated decreased degrees of stress hormone, cortisol, in sound volunteers (Schmidt et al., 2014). Interestingly, an ongoing report that regulated prebiotics (OSs that can advance the development of valuable commensal microscopic organisms) to solid volunteers brought about lower cortisol levels at arousing and improved thoughtfulness regarding positive upgrades contrasted with negative boosts in an enthusiastic order task and in a passionate acknowledgment task (Dash, Clarke, Berk, & Jacka, 2015). Overall, these investigations in sound people give away from of a connection among microbiota and passionate preparing; nonetheless, until now, clinical examinations concentrated on microbiota in mental scatters have just thought about despondency, hepatic encephalopathy, chemical imbalance range issue (ASD), and at times, tension-related manifestations associated with other ailments.

Despite the fact that downturn is a complex incessant disposition issue related to numerous components impacting its etiology, including hereditary qualities on earth, it has as of late been connected to modifications of the gut microbiota. Depression is normally depicted as a state-of-mind issue in which discouraged temperament as well as loss of intrigue or delight in life exercises is available at any rate over a time of 14 days. What is more, it must be joined by in any event five extra side effects that cause clinically critical debilitation in social, work, or other significant territories of working, for example, huge unexpected weight reduction or increase, a sleeping disorder or a lot of rest, weakness or loss of vitality, sentiments of uselessness or exorbitant blame, lessened capacity to think or concentrate, and intermittent musings of death (Naseribafrouei, Hestad, & Avershina, 2014). It is recommended that the gut microbiota influence the mind by means of the humoral and neural instrument of the gut-cerebrum pivot, with specific consideration on the vagus nerve (Naseribafrouei et al., 2014). Though a few connections between modified gut microbiota and sorrow have been built up in creature models, hardly any clinical examinations have been directed. In any case, one examination that did explicitly look at microbiome modifications and wretchedness in people by the investigation of fecal microbiota of 37 patients determined to have burdensome confusion contrasted with 18 nondepressed controls found numerous critical correlations (Bangsgaard Bendtsen, Krych, & Sørensen, 2012). The most articulated outcome was a general underrepresentation of Bacteroidetes in those determined to have despondency. All the more explicitly, *Alistipes*, a variety in the phylum of Bacteroidetes, was overrepresented in discouraged patients. Curiously, it has been accounted for in Balb/C mice to show a checked increment after a time of stress. *Alistipes* is likewise overrepresented in ceaseless weariness syndrome 56 and in touchy entrail condition (IBS), (Bangsgaard Bendtsen et al., 2012) proposing a potential normal element in a few issues that have comorbid nervousness and sadness. It has been recommended that *Alistipes* specifically is related to irritation and in this manner conceivably connected to discouragement through incendiary pathways (Bangsgaard Bendtsen et al., 2012). Of note, it has been indicated that *Alistipes* levels and other gut microbiota can be adjusted through dietary intervention (David, Maurice, & Carmody, 2014), which may demonstrate as a powerful mediation for burdensome clutters.

Uneasiness and state-of-mind issue are frequently comorbid with other ailments and in a few investigations that look at comorbid tension, a connection among microbiota and mental manifestations is watched. For instance, the organization of the probiotic, *L. casei* Shirota, diminished nervousness in patients with constant exhaustion syndrome (Rao,

Bested, & Beaulne, 2009). Anxiety and wretchedness are exceptionally comorbid in people with IBS (Fond, Loundou, & Hamdani, 2014). In IBS patients with clinically noteworthy uneasiness, everyday treatment with a prebiotic GOS blend for about a month decreased tension scores and had a critical constructive effect on nature of life (Silk, Davis, Vulevic, Tzortzis, & Gibson, 2009). In one investigation, a higher Firmicutes:Bacteroides proportion in IBS patients was corresponded with clinically huge sorrow and anxiety (Jeffery, O'Toole, & Öhman, 2012). In an increasingly broad sense, higher tension scores in IBS patients, and sound controls were related with lower fecal microbial decent variety supporting a connection among microbiota and mental state (Sundin, Rangel, Kumawat, Hultgren-Hornquist, & Brummer, 2014). More examinations considering the job microbiota in clinical populaces with tension issue, disposition issue, and comorbid mental manifestations are required. Porto-fundamental encephalopathy (PSE), otherwise called hepatic encephalopathy, is a neuropsychiatric condition related with modified gut microbiota. Its indications incorporate a fluctuating degree of cognizance, disarray, and movement to trance state. In serious cases, it can likewise prompt death (Cash et al., 2010). PSE is because of an aggregation of gut-inferred poisons in the circulatory system that are typically expelled by liver—in this manner brought about by hepatocellular disappointment or gateway fundamental venous shunting (Garcovich, Zocco, Roccarina, Ponziani, & Gasbarrini, 2012). Both these causes would permit the guessed nitrogen-containing poisons, for example, smelling salts, accepted to be produced by gut microorganisms from food, to enter the circulatory system and cross through the blood–cerebrum hindrance. At the point when these poisons cross into the cerebrum, nonetheless, they can cause the side effects recently referenced with respect to PSE. PSE is analyzed by the nearness of debilitated liver capacity and a prohibition of any substitute clarification of side effects (McGee, Bakens, Wiley, Riordan, & Webster, 2011). The impacts of PSE are reversible with medicines that work by stifling the creation of poisonous substances in the digestive tract, and as of now, most of these remedial choices being used incorporate prebiotics, intestinal medicines, and antibiotics (Garcovich et al., 2012). Prebiotics act by straightforwardly animating the development of bacterial strains conceivably valuable to have like *Bifidobacteria* and *Lactobacilli*, along these lines lessening the number and impacts of possibly progressively unsafe poison delivering inhabitant microbiota. One Cochrane meta-investigation of 550 randomized members more than seven preliminaries reasoned that while obviously probiotics can diminish plasma alkali focus when contrasted and fake treatment or no mediation, probiotics have given some advantage in improving clinical PSE results, albeit further randomized clinical preliminaries are needed. ASD is one more neurodevelopmental issue in which the job of microbiota is vital and another neurodevelopmental issue at the frontline of neuroscience and youngster psychiatry research. ASD incorporates neurodevelopmental clutters that are characterized by conduct perceptions, specifically dysfunctions in social collaboration and correspondence skills. The fundamental reasons for ASD are not yet explained; however, it is comprehended that both hereditary and ecological variables play a role. An ongoing survey paper considering the job of gut microbiota in the etiology of ASD found that numerous examinations have to be sure recognized the microbiota synthesis in ASD subjects to vary from those in sound controls.

For example, Clostridium microorganisms have been seen reliably as fundamentally raised in those with ASD (Finegold, Molitoris, & Song, 2002; Parracho, Bingham, Gibson, & McCartney, 2005; Song, Liu, & Finegold, 2004). On the other hand, there are some clashing patterns found as to some different strains of gut microbiota discoveries. For example, Bacteroidetes have been seen both as higher (Wang et al., 2011) and lower (Williams, Hornig, & Buie, 2011) in ASD subjects. Other potential markers recommending a job for gut microbiota have been found in an examination that announced higher fecal microscopic organisms aging item, higher smelling salts focuses, and higher short-chain unsaturated fat (SCFA) levels in 23 youngsters with ASD when contrasted with the 9 controls (Wang et al., 2012). Further proof of the job of the microbiota in ASD is introduced by the perception that intercessions with anti-infection agents and probiotics have prompted an improvement of conduct and correspondence in ASD subjects. Looking forward, mediations that focus on the microbial parity might be powerful in the treatment of neuropsychiatric condition

11 Metabolic disorder

A few creature and clinical investigations in the previous decade have exhibited that changes in gut microbiota may add to the advancement of obesity (Clarke, Murphy, & O'Sullivan, 2013). Differences in the decent variety and organization of microbiota in stout people have been reported (Bäckhed, Ding, & Wang, 2004; Ley et al., 2012). Normal intestinal greenery in people comprises a few phyla, including Firmicutes and Bacteroidetes, representing around 90% of all microbiota in the human intestine (Eckberg, Bik, & Bernstein, 2005). There is a rising speculation that gut microbiota is a modulator for the large genotype by method of an expansion in the Firmicutes/Bacteroidetes (F:B) proportion. Supporting this speculation, one examination looked at the dietary admission, fecal SCFA focuses, and gut microbial profiles in 52 fit [weight file (BMI) under 25] and 42 overweight or large (BMI more than 25) members, finding a positive connection between the blend of the log (F:B) proportion and SCFAs with one's BMI. These outcomes were recreated in a different report that recorded fecal

SCFA and F:B proportions in 11 lean and 11 overweight or hefty people. It was comparably discovered that overweight or large people had a higher relative Firmicutes bounty, a higher F:B proportion, and expanded fecal SCFA. These outcomes are steady with the theory that overweight or stout people produce more colonic SCFA than lean people because of the distinctions in colonic microbiota (Kamada et al., 2013).

There are a few potential instruments identified with how gut microbiota may influence digestion, for example, through the guideline of GI hormones, the creation of nutrients, help in the breakdown and processing of supplements, and fat storage which all lead to the expanded effectiveness of caloric extraction from food.

12 Gastro Intestinal Issue

Gut microbial irregularity has additionally been connected to many GI tract issues. IBS is related to adjustments in the gut microbiota and is frequently comorbid with nervousness disorders (Fond et al., 2014; Sartor, 2006; Gevers, Kugathasan, & Denson, 2014; Andoh et al., 2014; Torpy & Golub, 2011). Syndrome (IBS) is another normal GI malady influencing about 20% of people in the created world sooner or later in their lives (Kim, Deepinder, & Morales, 2012). It is an interminable issue that influences the internal organ and generally causes squeezing, stomach torment, swelling, gas, loose bowels, and stoppage. In spite of the fact that it is realized that the muscles of the intestinal dividers either contract too firmly causing gas, swelling, and loose bowels or too pitifully causing moderate food entry and dry stools, it is not recognized what legitimately causes these compression changes. In spite of the fact that components, for example, food bigotry, stress, and hormones are accepted to assume a job, Irritable Bowel Syndrome (IBS) is a GI issue most firmly identified with adjusted gut microbiota (Barrett et al., 2012). Indeed, an examination utilizing pyrosequencing to inspect the fecal microbiota profiles of 37 IBS patients age- and sex-coordinated to 20 controls found that the microbial mark might be identified with the clinical phenotype in a subset of IBS patients. Specifically, an expansion in Firmicutes-related taxa and an exhaustion of Bacteroidetes-related taxa best describe IBS subjects from the ordinary population (Barrett et al., 2012). Similarly, an examination analyzing the bacterial societies of 320 subjects found an immediate connection among IBS and an abundance microbiota (Torpy and Golub, 2011). The investigation found that of those patients with IBS, 37.5% were certain for small digestive tract bacterial excess (SIBO), contrasted with under 10% without IBS. What is more, this excess of microscopic organisms in the small digestive tract was increasingly pervasive in those with more serious runs overwhelming adaptation of this disease (Vrieze, Van Nood, & Holleman, 2012). This examination additionally found a more noteworthy number of types of microorganisms in IBS patients, affirming a key job of gut microbiota in IBS.

13 Regulation of microbiota and possibilities for treatment

Understanding the significant job that gut microbiota play in well-being, it is consoling that gut microbiota can surely be adjusted as a possibility for treatment in an assortment of microbiota-related maladies. In spite of the fact that the utilization of probiotics and anti-infection agents has been made broadly in creature concentrates as to neuropsychological scatters, for example, nervousness and sadness, there is constrained examination concerning their belongings in clinical populaces. In any case, there is expanding proof that probiotics might be valuable by diminishing burdensome and uneasiness like manifestations. For instance, it was found, in a twofold visually impaired, randomized clinical preliminary, that solid subjects who were given a blend of probiotics, containing *L. helveticus* and *B. longum*, for 30 days exhibited essentially less mental pain, as estimated by different surveys, than their coordinated partners on fake treatment control. Similarly, another twofold visually impaired, fake treatment controlled preliminary exhibited that solid subjects who scored in the lower third for discouraged disposition indicated critical improvement, in the wake of being taken care of a probiotic-containing milk drink for 3 weeks, when contrasted with their partners on fake treatment control (Moran & Shanahan, 2014). Gut microbiota modifications have likewise been accounted for in a considerable number of youngsters with mental imbalance, especially referring to a 10-crease increment in Clostridium numbers just as more noteworthy diversity (Critchfield, van Hemert, Ash, Mulder, & Ashwood, 2011). It is imagined that the organization of probiotic microorganisms to address changes in the microbiota might have the option to lessen irritation, reestablish epithelial boundary work, and possibly enhance conduct side effects related to kids with autism (Critchfield et al., 2011). Though the clinical examinations connecting the restorative effect of probiotics on neuropsychological disarranges are constrained in the beginning times, their outcomes support those from preclinical investigations, proposing that the balance of microbiota may in reality be focused for their remedial potential in neuropsychological scatters. It has additionally been accounted for that changed gut microbiota extents may add to the improvement of heftiness with various speculations and metabolic pathways proposed. The tweak of gut microbiota in overweight or large people with probiotics is an appealing idea as an approach to battle weight and related disorders. Modulation of the gut microbiota can be completed by different techniques, including probiotics, anti-infection agents, and fecal microbiota transplant. Regulation

of microbiota by probiotics has demonstrated viable in both preclinical and clinical preliminaries—in diet-initiated corpulent mice, the individuals who were treated with *Lactobacillus curvatus* and *Lactobacillus plantarum* demonstrated decreased weight gain just as a downregulation of proinflammatory qualities in fat tissue.

An investigation in sound people with a lot of instinctive fat indicated that devouring aged milk-containing *Lactobacillus gasseri* for 12 weeks was related with a decrease in BMI, despite the fact that discontinuance of utilization leads to fractional inversion of impacts, recommending that persistent utilization might be required to keep up gainful effects (Nøhr, 2013). Both these examinations show the expected advantages of probiotics in corpulence treatment. Antitoxins, for example, vancomycin, have likewise demonstrated powerful. When presented in mice, vancomycin has essentially diminished the extents of Firmicutes and Bacteroidetes and expanded Proteobacteria, bringing about decreased weight gain and improved fasting glucose and triglyceride levels. Gut microbiota transplants through fecal microbiota transplants may likewise demonstrate successful—in a little report a mixture of microbiota from lean givers to patients with metabolic disorder expanded their insulin affectability when estimated at about a month and a half after implantation, supporting in weight loss (Barrett et al., 2012).

14 Conclusion

The more the exploration uncovers, the more prominent the influence gut microbiota seems to have, at about all frameworks and levels of the human body. Of course, these clinical ailments further represent the imperative pretended by gut microbiota in the human body, both through solid, gainful states and, all the more critically, in changed, broken states. The previously mentioned examinations, which center around the effect of gut microbiota on the host, are fundamental to our understanding the impact of these frameworks. In the nonappearance or brokenness of ordinary gut greenery, a large number of illnesses may happen, revealing insight into the significant job kept up by the gut-cerebrum hub. Further examinations with center around strain, dose, span of treatment, and nature of mental issue are justified to decide probiotics' adequacy for improving emotional well-being. Toward the end, the brilliant key to respond to these inquiries is to study and think about the synthesis of gut microbiota utilizing subatomic techniques in solid and patient people and furthermore when probiotic mediations.

References

Adinoff, B. (1992). Vagal tone decreases following intravenous diazepam. *Psychiatry Research*, *41*, 89–97.

Alenghat, T., & Artis, D. (2014). Epigenomic regulation of host–microbiota interactions. *Trends in Immunology*, *35*, 518–525.

Anderberg, R. H. (2014). Dopamine signaling in the amygdala, increased by food ingestion and GLP-1, regulates feeding behavior. *Physiology & Behavior*, *136*, 135–144.

Andoh, A., Kobayashi, T., Kuzuoka, H., Tsujikawa, T., Suzuki, Y., Hirai, F., et al. (2014). Characterization of gut microbiota profiles by disease activity in patients with Crohn's disease using data mining analysis of terminal restriction fragment length polymorphisms. *Biomedical Reports*, *2*(3), 370–373.

Bäckhed, F., Ding, H., Wang, T., et al. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(44), 15718–15723.

Bangsgaard Bendtsen, K. M., Krych, L., Sørensen, D. B., et al. (2012). Gut microbiota composition is correlated to grid floor induced stress and behavior in the BALB/c mouse. *PLoS One*, *7*(10), e46231.

Banks, W. A., & Erickson, M. A. (2010). The blood–brain barrier and immune function and dysfunction. *Neurobiology of Disease*, *37*(1), 3726–3732.

Barrett, E., Ross, R. P., O'Toole, P. W., Fitzgerald, G. F., & Stanton, C. (2012). Gamma aminobutyric acid production by culturable bacteria from the human intestine. *Journal of Applied Microbiology*, *113*(2), 411–417.

Bercik, P. (2010). Chronic gastrointestinal inflammation induces anxiety-like behavior and alters central nervous system biochemistry in mice. *Gastroenterology*, *139*, 2102–2112.

Bercik, P. (2011). The intestinal microbiota affects central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology*, *141*, 599–609.

Bercik, P., Collins, S. M., & Verdu, E. F. (2012). Microbes and the gut-brain axis. *Neurogastroenterology & Motility*, *24*, 405–413.

Bode, L. (2004). Inhibition of monocyte, lymphocyte, and neutrophil adhesion to endothelial cells by human milk oligosaccharides. *Thrombosis and Haemostasis*, *92*, 1402–1410.

Cani, P. D. (2005). Oligofructose promotes satiety in rats fed a high-fat diet: Involvement of glucagon-like peptide-1. *Obesity Research*, *13*, 1000–1007.

Cash, W. J., McConville, P., McDermott, E., McCormick, P. A., Callender, M. E., & McDougall, N. I. (2010). Current concepts in the assessment and treatment of hepatic encephalopathy. *QJM*, *103*(1), 9–16.

Chu, H., & Mazmanian, S. K. (2013). Innate immune recognition of the microbiota promotes host-microbial symbiosis. *Nature Immunology*, *14*, 668–675.

Clarke, S. F., Murphy, E. F., O'Sullivan, O., et al. (2013). Targeting the microbiota to address diet-induced obesity: A time dependent challenge. *PLoS One*, *8*(6), e65790.

Collins, S. M., & Bercik, P. (2009). The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease. *Gastroenterology*, *136*, 2003–2014.

Critchfield, J. W., van Hemert, S., Ash, M., Mulder, L., & Ashwood, P. (2011). The potential role of probiotics in the management of childhood autism spectrum disorders. *Gastroenterology Research and Practice*, *2011*, 161358.

Dash, S., Clarke, G., Berk, M., & Jacka, F. N. (2015). The gut microbiome and diet in psychiatry: Focus on depression. *Current Opinion in Psychiatry*, *28*(1), 1–6.

David, L. A., Maurice, C. F., Carmody, R. N., et al. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, *505*(7484), 559–563.

De Lartigue, G. (2011). Vagal afferent neurons in high fat diet-induced obesity; intestinal microflora, gut inflammation and cholecystokinin. *Physiology & Behavior*, *105*, 100–105.

de Vries, H. E. (1996). The influence of cytokines on the integrity of the blood–brain barrier in vitro. *Journal of Neuroimmunology*, *64*, 37–43.

Dinan, T. G. (2015). Collective unconscious: How gut microbes shape human behavior. *Journal of Psychiatric Research*, *63*, 1–9.

Dinan, T. G., & Cryan, J. F. (2012). Regulation of the stress response by the gut microbiota: Implications for psychoneuroendocrinology. *Psychoneuroendocrinology*, *37*(9), 1369–1378.

Dockray, G. J. (2014). Gastrointestinal hormones and the dialogue between gut and brain. *The Journal of Physiology*, *592*, 2927–2941.

Donato, K. A. (2010). *Lactobacillus rhamnosus* GG attenuates interferon-γ and tumour necrosis factor-α-induced barrier dysfunction and pro-inflammatory signalling. *Microbiology*, *156*, 3288–3297.

Dowlati, Y. (2010). A meta-analysis of cytokines in major depression. *Biological Psychiatry*, *67*, 446–457.

Eckberg, P. B., Bik, E. M., Bernstein, C. N., et al. (2005). Diversity of the human intestinal microbial flora. *Science*, *308*(5728), 1635–1638.

Eiwegger, T. (2010). Prebiotic oligosaccharides: In vitro evidence for gastrointestinal epithelial transfer and immunomodulatory properties. *Pediatric Allergy and Immunology*, *21*, 1179–1188.

Ek, M. (2001). Inflammatory response: Pathway across the blood–brain barrier. *Nature*, *410*, 430–431.

Erny, D. (2015). Host microbiota constantly control maturation and function of microglia in the CNS. *Nature Neuroscience*, *18*, 965–977.

Finegold, S. M., Molitoris, D., Song, Y., et al. (2002). Gastrointestinal microflora studies in late-onset autism. *Clinical Infectious Diseases*, *35*(Suppl. 1), S6–S16.

Fond, G., Loundou, A., Hamdani, N., et al. (2014). Anxiety and depression comorbidities in irritable bowel syndrome (IBS): A systematic review and metaanalysis. *European Archives of Psychiatry and Clinical Neuroscience*, *264*(8), 651–660.

Fung, T. C., Olson, C. A., & Hsiao, E. Y. (2017). Interactions between the microbiota, immune and nervous systems in health and disease. *Nature Neuroscience*, *20*, 145–155.

Gagliano, H. (2014). High doses of the histone deacetylase inhibitor sodium butyrate trigger a stress-like response. *Neuropharmacology*, *79*, 5–82.

Garcovich, M., Zocco, M. A., Roccarina, D., Ponziani, F. R., & Gasbarrini, A. (2012). Prevention and treatment of hepatic encephalopathy: Focusing on gut microbiota. *World Journal of Gastroenterology*, *18*(46), 6693–6700.

Gevers, D., Kugathasan, S., Denson, L. A., et al. (2014). The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host & Microbe*, *15*(3), 382–392.

Heldt, S. A. (2007). Hippocampus-specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories. *Molecular Psychiatry*, *12*, 656–670.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., et al. (2014). Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*, *11*, 506–514.

Horn, T., & Klein, J. (2013). Neuroprotective effects of lactate in brain ischemia: Dependence on anesthetic drugs. *Neurochemistry International*, *62*, 251–257.

Jasarevic, E., Howerton, C. L., Howard, C. D., & Bale, T. L. (2015). Alterations in the vaginal microbiome by maternal stress are associated with metabolic reprogramming of the offspring gut and brain. *Endocrinology*, *156*, 3265–3276.

Jeffery, I. B., O'Toole, P. W., Öhman, L., et al. (2012). An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. *Gut*, *61*(7), 997–1006.

Kamada, N., Seo, S. U., Chen, G. Y., & Nunez, G. (2013). Role of the gut microbiota in immunity and inflammatory disease. *Nature Reviews Immunology*, *13*, 321–335.

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry*, *62*, 593–602.

Kim, G., Deepinder, F., Morales, W., et al. (2012). *Methanobrevibacter smithii* is the predominant methanogen in patients with constipation-predominant IBS and methane on breath. *Digestive Diseases and Sciences*, *57*(12), 3213–3218.

Kim, K. S. (2016). Dietary antigens limit mucosal immunity by inducing regulatory T cells in the small intestine. *Science*, *351*, 858–863.

Kirchner, A. (2000). Left vagus nerve stimulation suppresses experimentally induced pain. *Neurology*, *55*, 1167–1171.

Leung, K., & Thuret, S. (2015). Gut microbiota: A modulator of brain plasticity and cognitive function in ageing. *Healthcare*, *3*, 898–916.

Ley, R. E., Backhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., Gordon, J. I., et al. (2012). Does the human gut microbiota contribute to the etiology of autism spectrum disorders? *Digestive Diseases and Sciences*, *57*(8), 1987–1989.

Louveau, A. (2015). Structural and functional features of central nervous system lymphatic vessels. *Nature*, *523*, 337–341.

Lu, Y. (2008). BDNF: A key regulator for protein synthesis-dependent LTP and long-term memory? *Neurobiology of Learning and Memory*, *89*, 312–323.

Lyte, M. (2011). Probiotics function mechanistically as delivery vehicles for neuroactive compounds: Microbial endocrinology in the design and use of probiotics. *Bioessays*, *33*, 574–581.

Mackey, D., & McFall, A. J. (2006). MAMPs and MIMPs: Proposed classifications for inducers of innate immunity. *Molecular Microbiology*, *61*, 1365–1371.

Martinowich, K., & Lu, B. (2008). Interaction between BDNF and serotonin: Role in mood disorders. *Neuropsychopharmacology*, *33*, 73–83.
Mayer, E. A., Knight, R., Mazmanian, S. K., Cryan, J. F., & Tillisch, K. (2011). Gut microbes and the brain: Paradigm shift in neuroscience. *The Journal of Neuroscience*, *34*, 15490–15496.
McCusker, R. H., & Kelley, K. W. (2013). Immune–neural connections: How the immune system's response to infectious agents influences behavior. *The Journal of Experimental Biology*, *216*, 84–98.
McGee, R. G., Bakens, A., Wiley, K., Riordan, S. M., & Webster, A. C. (2011). Probiotics for patients with hepatic encephalopathy. *Cochrane Database of Systematic Reviews*, *11*, CD008716.
McNutt, M. D. (2012). Neurobehavioral effects of interferon-α in patients with hepatitis C: Symptom dimensions and responsiveness to paroxetine. *Neuropsychopharmacology*, *37*, 1444–1454.
Messaoudi, M., Lalonde, R., Violle, N., et al. (2011a). Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects. *British Journal of Nutrition*, *105*(5), 755–764.
Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., et al. (2011b). Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects. *British Journal of Nutrition*, *105*, 755–764.
Mezzacappa, E. S. (2001). Vagal rebound and recovery from psychological stress. *Psychosomatic Medicine*, *63*, 650–657.
Miller, A. H. (2013). Cytokine targets in the brain: Impact on neurotransmitters and neurocircuits. *Depression and Anxiety*, *30*, 297–306.
Moran, C. P., & Shanahan, F. (2014). Gut microbiota and obesity: Role in aetiology and potential therapeutic target. *Best Practice & Research: Clinical Gastroenterology.*, *28*(4), 585–597.
Moretti, M. (2011). Behavioral and neurochemical effects of sodium butyrate in an animal model of mania. *Behavioural Pharmacology*, *22*, 766–772.
Morris, G. (2016). The role of the microbial metabolites including tryptophan catabolites and short chain fatty acids in the pathophysiology of immune-inflammatory and neuroimmune disease. *Molecular Neurobiology*, *2016*, 763–781. http://dx.doi.org/10.1007/s12035-016-0004-2 Published online June 27.
Morris, G. L., & Mueller, W. M. (1999). Long-term treatment with vagus nerve stimulation in patients with refractory epilepsy. *Neurology*, *53*, 1731–1735.
Moustafa, A., Li, W., Anderson, E. L., Wong, E. H. M., Dulai, P. S., Sandborn, W. J., et al. (2018). Genetic risk, dysbiosis, and treatment stratification using host genome and gut microbiome in inflammatory bowel disease. *Clinical and Translational Gastroenterology*, *9*, e132.
Naseribafrouei, A., Hestad, K., Avershina, E., et al. (2014). Correlation between the human fecal microbiota and depression. *Neurogastroenterology & Motility*, *26*(8), 1155–1162.
Nøhr, M. K. (2013). GPR41/FFAR3 and GPR43/FFAR2 as cosensors for short-chain fatty acids in enteroendocrine cells vs FFAR3 in enteric neurons and FFAR2 in enteric leukocytes. *Endocrinology*, *154*, 3552–3564.
O'Hara, A. M. (2006). Functional modulation of human intestinal epithelial cell responses by *Bifidobacterium infantis* and *Lactobacillus salivarius*. *Immunology*, *118*, 202–215.
Overduin, J. (2013). Dietary galacto-oligosaccharides and calcium: Effects on energy intake, fat-pad weight and satiety-related, gastrointestinal hormones in rats. *British Journal of Nutrition*, *109*, 1338–1348.
Palmer, C., Bik, E. M., DiGiulio, D. B., Relman, D. A., & Brown, P. O. (2007). Development of the human infant intestinal microbiota. *PLoS Biology*, *5*, e177.
Parracho, H. M., Bingham, M. O., Gibson, G. R., & McCartney, A. L. (2005). Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. *Journal of Medical Microbiology*, *54*(Pt 10), 987–991.
Perry, R. J. (2016). Acetate mediates a microbiome–brain–β-cell axis to promote metabolic syndrome. *Nature*, *534*, 213–217.
Pessi, T. (2000). Interleukin-10 generation in atopic children following oral *Lactobacillus rhamnosus* GG. *Clinical & Experimental Allergy*, *30*, 1804–1808.
Psichas, A. (2015). The short chain fatty acid propionate stimulates GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents. *International Journal of Obesity*, *39*, 424–429.
Qin, J. (2010a). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, *464*, 59–65.
Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., et al. (2010b). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, *464*, 59–65.
Rao, A. V., Bested, A. C., Beaulne, T. M., et al. (2009). A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. *Gut Pathogens*, *1*(1), 6.
Ray, A. (2015). Gut microbial dysbiosis due to helicobacter drives an increase in marginal zone B cells in the absence of IL-10 signaling in macrophages. *Journal of Immunology*, *195*, 3071–3085.
Redhu, N. (2016). O-005 YI microbiota drives inflammation by altering intestinal lamina propria macrophage phenotype in a novel IL10R-deficient model of very early onset IBD. *Inflammatory Bowel Disease*, 22.
Rush, A. J. (2005). Vagus nerve stimulation for treatment-resistant depression: A randomized, controlled acute phase trial. *Biological Psychiatry*, *58*, 347–354.
Sackeim, H. A. (2001). Vagus nerve stimulation (VNS™) for treatment-resistant depression: Efficacy, side effects, and predictors of outcome. *Neuropsychopharmacology*, *25*, 713–728.
Salam, O. M. A. (2004). Fluoxetine and sertraline stimulate gastric acid secretion via a vagal pathway in anaesthetized rats. *Pharmacological Research*, *50*, 309–316.
Sartor, R. B. (2006). Mechanisms of disease: Pathogenesis of Crohn's disease and ulcerative colitis. *Nature Clinical Practice Gastroenterology & Hepatology*, *3*(7), 390–407.
Schmidt, K., Cowen, P. J., Harmer, C. J., Tzortzis, G., Errington, S., & Burnet, P. W. (2014). Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. *Psychopharmacology (Berlin)*, *232*(10), 1793–1801. Epub.

Selhub, E. M., Logan, A. C., & Bested, A. C. (2014). Fermented foods, microbiota, and mental health: Ancient practice meets nutritional psychiatry. *Journal of Physiological Anthropology*, *33*, 2. doi: 10.1186/18806805-33-2.

Silk, D. B., Davis, A., Vulevic, J., Tzortzis, G., & Gibson, G. R. (2009). Clinical trial: The effects of a trans-galactooligosaccharide prebiotic on faecal microbiota and symptoms in irritable bowel syndrome. *Alimentary Pharmacology & Therapeutics*, *29*(5), 508–518.

Smith, R. (2014). Increased association over time between regional frontal lobe BOLD change magnitude and cardiac vagal control with sertraline treatment for major depression. *Psychiatry Research: Neuroimaging*, *224*, 225–233.

Song, Y., Liu, C., & Finegold, S. M. (2004). Real-time PCR quantitation of clostridia in feces of autistic children. *Applied and Environmental Microbiology*, *70*(11), 6459–6465.

Spalding, T. W. (2000). Vagal and cardiac reactivity to psychological stressors in trained and untrained men. *Medicine & Science in Sports & Exercise*, *32*, 581–591.

Stadlbauer, U. (2015). PYY 3-36: Beyond food intake. *Frontiers in Neuroendocrinology*, *38*, 1–11.

Stilling, R. M. (2014). Microbial genes, brain & behavior–epigenetic regulation of the gut–brain axis. *Genes, Brain and Behavior*, *13*, 69–86.

Sun, J. (2016). Antidepressant-like effects of sodium butyrate and its possible mechanisms of action in mice exposed to chronic unpredictable mild stress. *Neuroscience Letters*, *618*, 159–166.

Sundin, J., Rangel, I., Kumawat, A. K., Hultgren-Hornquist, E., & Brummer, R. J. (2014). Aberrant mucosal lymphocyte number and subsets in the colon of postinfectious irritable bowel syndrome patients. *Scandinavian Journal of Gastroenterology*, *49*(9), 1068–1075.

Thayer, J. F., & Sternberg, E. M. (2009). Neural concomitants of immunity: Focus on the vagus nerve. *Neuroimage*, *47*, 908–910.

Tillisch, K., Labus, J., Kilpatrick, L., et al. (2013a). Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology*, *144*(7), e1391–e1394.

Tillisch, K., Labus, J., Kilpatrick, L., Jiang, Z., Stains, J., Ebrat, B., et al. (2013b). Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology*, *144*, 1394–1401.

Torpy, J. M., & Golub, R. M. (2011). JAMA patient page. Irritable bowel syndrome. *JAMA*, *306*(13), 1501.

Udina, M. (2012). Interferon-induced depression in chronic hepatitis C: A systematic review and meta-analysis. *The Journal of Clinical Psychiatry*, *73*, 1128–1138.

Vrieze, A., Van Nood, E., Holleman, F., et al. (2012). Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology*, *143*(4), 913–916.e7.

Wang, F. (2006). IFN-γ-induced TNFR2 expression is required for TNF-dependent intestinal epithelial barrier dysfunction. *Gastroenterology*, *131*, 1153–1163.

Wang, L., Christophersen, C. T., Sorich, M. J., Gerber, J. P., Angley, M. T., & Conlon, M. A. (2011). Low relative abundances of the mucolytic bacterium *Akkermansia muciniphila* and *Bifidobacterium* spp. in feces of children with autism. *Applied and Environmental Microbiology*, *77*(18), 6718–6721.

Wang, L., Christophersen, C. T., Sorich, M. J., Gerber, J. P., Angley, M. T., & Conlon, M. A. (2012). Elevated fecal short chain fatty acid and ammonia concentrations in children with autism spectrum disorder. *Digestive Diseases and Sciences*, *57*(8), 2096–2102.

Wikoff, W. R. (2015). Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(2009), 3698–3703.

Williams, B. L., Hornig, M., Buie, T., et al. (2011). Impaired carbohydrate digestion and transport and mucosal dysbiosis in the intestines of children with autism and gastrointestinal disturbances. *PLoS One*, *6*(9), e24585.

Yamagishi, K., Doe, N., Sumida, M., Watanabe, Y., Yoshida, M., Yamamoto, H., et al. (2015). Hepatocyte nuclear factor 4 alpha is a key factor related to depression and physiological homeostasis in the mouse brain. *PLoS One*, *10*, 0119021. doi: 10.1371/journal.pone.0119021.

Zhou, W. (2015). Protective effects of bifidobacterial on intestines in newborn rats with necrotizing enterocolitis and its regulation on TLR2 and TLR4. *Genetics and Molecular Research*, *14*, 11505–11514.

Chapter 14

Probiotics, Diet, and Gut Microbiome Modulation in Metabolic Syndromes Prevention

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1 Metabolic syndrome

Metabolic syndrome (MetS), also called insulin resistant syndrome, MetS X or dysmetabolic syndrome, refers to a complex health condition that encompasses an aggregate of three or more risk factors [such as high blood pressure, abdominal obesity, elevated triglyceride (TG) levels, low high-density lipoprotein (HDL) levels, and high fasting levels of blood sugar] that instigate the potential for cardiovascular diseases (CVDs) and type 2 diabetes mellitus (T2DM) to occur (Grundy, 2020; Shoushou et al., 2020; Zafar, Khaliq, Ahmad, Manzoor, & Lone, 2018). It is important to note that at least three of the five criteria must be present to characterize a patient as having MetS shown in Table 14.1 (Grundy, 2020; Saklayen, 2018; Shoushou et al., 2020). T2DM is associated with insulin resistance and a variety of cardiovascular conditions which can arise with having MetS (Saklayen, 2018; Shoushou et al., 2020).

It is worth noting that MetS is still an issue under study and thus there is no definitive way to define, since various definitions have been given by many authorities such as the World Health Organization (WHO), the International Diabetes Federation (IDF), and others. For the most part the underlying theme that seems to run through them are the following five risk factors: abdominal obesity, hyperglycemia, hypertriglyceridemia, low HDL cholesterol, and hypertension (Table 14.1). Although the etiology of malady is not fully understood, MetS is postulated to be correlated with insulin resistance, adipose tissue dysfunction, chronic inflammation, oxidative stress, circadian disruption, microbiota, genetic factors, and so on (He & Shi, 2017; Xu, Li, Adams, Kubena, & Guo, 2018).

People with MetS are thrice as likely to have a heart attack or stroke, and twice as likely to die compared to people without the syndrome (He & Shi, 2017). The malady is postulated to have been propagated by Western lifestyle but is now a global epidemic—an emerging yet mounting chronic plague of the 21st century (Green, Arora, & Prakash, 2020; Kassi, Pervanidou, Kaltsas, & Chrousos, 2011; Saklayen, 2018). The prevalence of MetS is one-fourth of the global population. That is, over a billion of people in the world have MetS (Saklayen, 2018). Though it is true that MetS is harder to measure, the high incidence of its resulting conditions T2DM and CVD strikes an awakening of the probable high numbers of MetS. As has already been established, it is impossible to talk about MetS in isolation without mentioning its affiliates (obesity, T2DM, and CVDs). However, this does not mean that MetS is synonymous to them. In 2017, Centers for Disease Control and Prevention (CDC) of the United States of America (USA) reported that about 30.2 million adults aged 18 years or older had T2DM. It was established that the incidence of T2DM increased with age, stretching to 25.2% among US seniors (65 years or older). Prevalence of prediabetes or MetS was about three times more, and thus about one-third of US adults have MetS. In addition, 15% of American Indians had a higher incidence of T2DM and South Asian American had an even higher prevalence of MetS along with higher incidence of abdominal obesity (Palaniappan, Wong, Shin, Fortmann, & Lauderdale, 2011). Whether these data are enough to establish that certain ethnic groups are more prone to MetS is a subject of debate and requires further detailed studies. In China the incidence of MetS increased from 8% to 10.6% in urban areas and 4.9% to 5.3% in rural

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00014-9
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TABLE 14.1 The figures matter-risk factors criteria for MetS.

	Blood pressure (mmHg)	HDL (mg/dL)	Triglyceride (mg/dL)	Fasting blood sugar (mg/dL)	Waist circumference (inches)
Male	>130/85	<40	>150	>110	>40
Female	>130/85	<50	>150	>110	>35

areas. Overweight and obesity cases have more than doubled across the world in both adults and children alike since 2015. Gone are the days where the issue of obesity was affiliated to the affluent. A global survey revealed a rise in the incidence of obesity from 1.1% in 1980 to 3.85% in 2015 (https://time.com/4813075/obesity-overweight-weight-loss). The highest increase in obesity cases occurred in young men (25–29 years) from developing countries. In all the Middle Eastern countries, prevalence of MetS was much higher among women than men. IDF speculates that incidence of diabetes is 8.8% (415 million) as of 2015 and is expected to increase to 10.4% (642 million) by 2040 across the globe. Though the prevalence is comparatively low in African countries, some of the highest growth rate in diabetes is expected to be in sub-Saharan Africa and Middle East/North Africa (141% and 104%, respectively, in the next 25 years). From all the incidences recorded, susceptibility increase with age (Grundy, 2020; Saklayen, 2018; Shoushou et al., 2020).

1.1 Metabolic syndrome and diet

Lifestyle factors such as sedentary habits, reduced physical activity due to mechanized transportations, busy schedules, and poor dietary habits like increased consumption of high calorie–low fiber fast foods have been shown to increase the risk of MetS (Green et al., 2020). Like its consequential outcomes, CVDs and T2DM, the risk of MetS can be reduced if appropriate actions are taken to reduce the occurrence of the risk factors already mentioned (high blood pressure, abdominal obesity, elevated TG levels, low HDL levels, and high fasting levels of blood sugar) (Fenwick et al., 2019; Grundy, 2020). It is true that factors like genetics give little room for any sort of alterations and there are pharmaceutical prescriptions that can be used to treat individual components of MetS (Bassi et al., 2014). However, changes in lifestyle habits (i.e., diet and exercise) have beneficial effects and represent the foundation of all treatments. Exercise and especially diet control are within the power and will of everyone. Everyone eats to stay alive and keep the body functioning as required. The link between diet and health cannot be overemphasized. It is a powerful tool that can have therapeutic effects on MetS indicators. Dietary recommendations that include fiber-rich, low-fat, high-protein, polyphenol-rich, and Mediterranean diets (MDiets) have been proposed to reduce MetS. Diets rich in fruits, vegetables, grains, fish, low-carbohydrates, and low-fat dairy products have a protective role, while limiting the consumption of other high-glycemic-index carbohydrates, saturated fats, processed foods is pivotal in normalizing metabolic anomalies, thereby reducing MetS (Von Bibra et al., 2014). Several studies have shown that low-glycemic index carbohydrates like whole grain and fiber are associated with a lower incidence of the MetS and improved insulin sensitivity, lower fasting, total and low-density lipoprotein (LDL) cholesterol (Di Daniele et al., 2013; Von Bibra et al., 2014). In effect, these approaches improve metabolism and promote overall health through high-quality diets (Fenwick et al., 2019). Dramatic changes in diet have proven immensely to alter the metabolic indicators of the MetS.

A famously popular diet that has stood out in the fight against MetS is the MDiet in Fig. 14.1. This diet is selective on the type, proportion, and nutritional quality of food that is used in its preparation. Evidence of the therapeutic effect of the diet date back decades (Table 14.2). A dietary intervention trial, comparing effects of different diets, revealed that the MDiet yielded the most positive changes in fasting plasma glucose and insulin levels among diabetics as well as an overall improvement in systolic and diastolic blood pressure (Di Daniele et al., 2013; Mitjavila et al., 2013). According to the American Heart Association, an MDiet is rich with a variety of fruits, vegetables, bread and other grains, potatoes, beans, nuts, and seeds; uses olive oil as a primary fat source; and low to moderately incorporates the use of dairy products, eggs, fish, and poultry. The preferable protein is fish and poultry rather than red meat. There is little to no use of processed foods that are likely to be high in sodium and/or fat (e.g., bacon, canned tuna) and wine may be consumed in low-to-moderate amounts, usually with the meal. For the sweet tooth's, any dessert after the meal is strictly fruits (Di Daniele et al., 2013; Mitjavila et al., 2013; Radd-Vagenas, Kouris-Blazos, Singh, & Flood, 2017). Table 14.2 shows human studies showing therapeutic effects of MDiet.

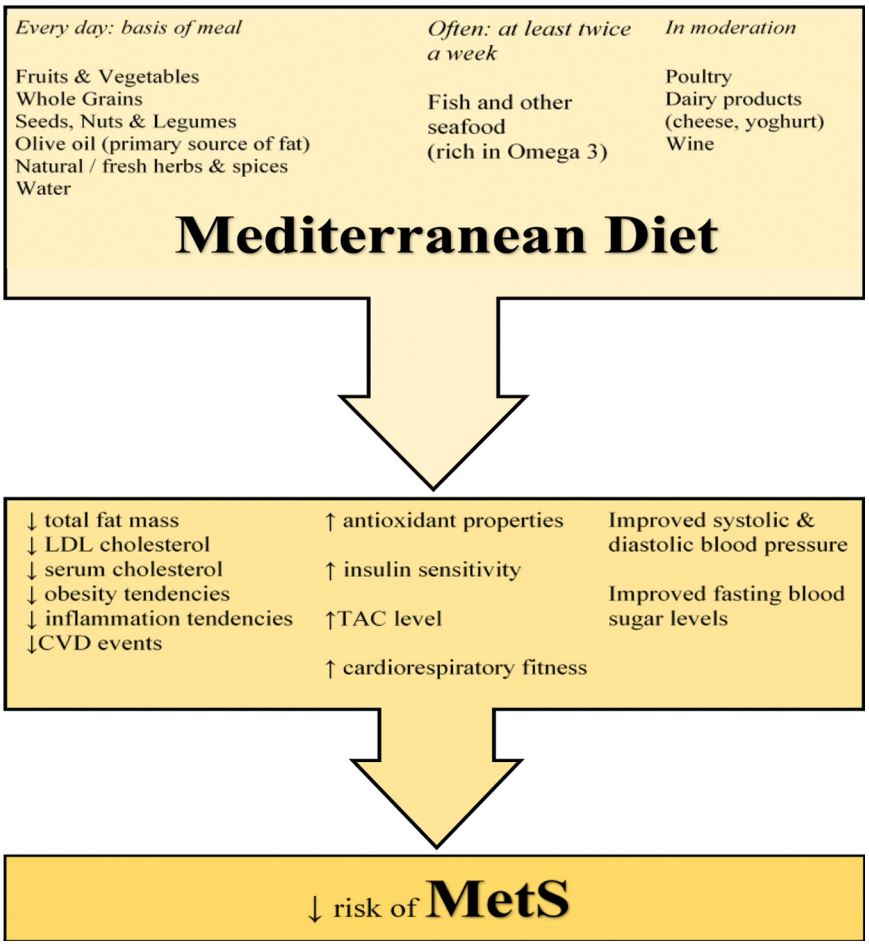


FIGURE 14.1 Benefits of Mediterranean diet on MetS. ↑, Increase; ↓, decrease; CVD, cardiovascular diseases; LDL, low-density lipoprotein; MetS, metabolic syndrome; TAC, total antioxidant capacity.

TABLE 14.2 Human studies showing therapeutic effects of MDiet.

Reference, country	Population	Age (years)	Follow-up	Purpose of the study	Clinical outcomes
Cuenca-García et al. (2014), Spain	12,449 Middle-aged healthy men and women	20–84	12 Years	The study examined the association between three predefined dietary indices (Ideal Diet Index, Diet Quality Index, and MDiet score) and both CVD risk factors and long-term mortality in adult Aerobics Center Longitudinal Study's participants	Higher Ideal Diet Index, Diet Quality Index, and Mediterranean Diet Score scores were significantly linked to lower body mass index, cholesterol and glucose levels, diastolic blood pressure, and higher cardiorespiratory fitness
De Lorenzo et al. (2001), Italy	19 Obese Italian women without obesity-related complications or other diseases	>32	2 Months	The study evaluated the efficacy and the safety of an MHMD on body composition and metabolic profile	Total fat mass and segmental fat mass from trunk and legs were significantly decreased. A significant decrease of basal insulin, total and LDL-cholesterol, uric acid, and fibrinogen was observed
Vessby et al. (2001), Sweden	162 Healthy subjects	30–65	3 Months	The aim was to evaluate whether a change in dietary fat quality could improve insulin action	A decrease of saturated fatty acid and an increase of monounsaturated fatty acid improve insulin sensitivity but have no effect on insulin secretion
Fitó et al. (2007), Spain	372 Subjects with high cardiovascular risk	55–80	3 Months	The aim was to verify the efficacy of the MDiet on the primary prevention of coronary heart disease in patients with high cardiovascular risk	After 3 months of MDiet, individuals at high cardiovascular showed significant reductions in cellular lipid levels and LDL oxidation

(*Continued*)

TABLE 14.2 Human studies showing therapeutic effects of MDiet. (*Cont.*)

Reference, country	Population	Age (years)	Follow-up	Purpose of the study	Clinical outcomes
Pitsavos et al. (2005), Greece	3042 People with no clinical evidence of cardiovascular disease	18–89	1 Year	"The ATTICA study" assessed the effect of the MDiet on total antioxidant capacity (TAC)	Greater adherence to the MDiet is associated to higher TAC levels and low oxidized LDL-cholesterol concentrations
Salas-Salvadó et al. (2011), Spain	7447 Men and women with no previously documented cardiovascular diseases	55–80	4.8 Years	PREDIMED randomized trial analyzed the effect of MDiet on incidence and reversion of metabolic syndrome	MDiet with extravirgin oil after a median follow-up of 4.8 years had reduced by 30% of the rate of CVD events. MDiet with nuts had reduced by 28%. MDiet either supplemented with extravirgin olive oil or nuts was not associated with the onset of metabolic syndrome but only with its regression
De Lorenzo et al. (2010), Italy	150 Caucasian and Italian men (100 healthy males and 50 males with CKD)	30–65	14 Days	To verify the effect of IMD on body composition and biochemical parameters in healthy individuals and in individuals with CKD, in order to decrease CVD risk factor and the progression of renal diseases	The IMOD diet reduced the total homocysteine, phosphorus, and albuminuria levels in individuals with CKD and reduce the cardiovascular risk profile in both populations examined in the study
Basu et al. (2010a), the United States	27 Subjects with metabolic syndrome	47 ± 3	2 Months	The aim was to verify if a freeze–dried strawberry supplementation can improve blood pressure, impaired glucose, dyslipidemia, or circulating adhesion molecules in obese subjects with metabolic syndrome	A short-term supplementation with 4 cups of freeze–dried strawberry beverage improve selected atherosclerotic risk factors, including dyslipidemia and circulating adhesion molecules in subjects with MetS
Basu et al. (2010b), the United States	48 Men and women with MetS	50 ± 3	2 Months	The study aimed to evaluate the effects of blueberry supplementation on features of metabolic syndrome, lipid peroxidation, and inflammation in obese men and women	The blueberry supplementation decreases systolic and diastolic blood pressures, whereas the serum glucose concentration and lipid profiles were not affected
Tierney et al. (2011), EU	417 MetS subjects	53 ± 2	3 Months	In LIPGENE study a large pan-European isocaloric dietary intervention study of MetS subjects, SFA have been replaced with MUFA or low-fat, high complex carbohydrate	Improvement of insulin sensitivity only in patients whose habitual preintervention dietary fat intake was below the median (<36% energy from fat)
Kolehmainen et al. (2012), Finland	27 Subjects with MetS	53 ± 6	2 Months	The aim was to study the impact of bilberries on inflammation and gene expression profile in peripheral blood mononuclear cells	Blueberry supplementation decreases serum high sensitivity C-reactive protein, IL-6, IL-12, and LPS concentration, demonstrating a decrease of cardiometabolic risk in long term
Di Daniele et al. (2013), Italy	80 White Italian subjects with MetS	48.7 ± 13	6 Months	The aim was to evaluate the benefits of dietary intervention based on a typical IMD on body composition, cardiometabolic changes and reduction in CVD in patients with MetS	The MetS was resolved in 52% of the patients. Significant improvements in systolic and diastolic blood pressure and fasting glucose occurred
Li et al. (2015), Taiwan	25 Young females with BMI ≥ 30 kg/m^2	20–30	2 Months	This study showed the effect of tomato juice's supplementation on indices linked to metabolic health and adipokine profiles in generally healthy people	Daily tomato juice supplementation reduces waist circumference, as well as serum cholesterol and inflammatory adipokine levels in young healthy women and these results are unrelated to body fat changes

BCM, Body cellular mass; *CKD*, chronic kidney disease; *CVD*, cardiovascular diseases; *HDL*, high-density lipoprotein; *IL-12*, interleukin-12; *IL-6*, interleukin-6; *IL-8*, interleukin-8; *IMD*, Italian Mediterranean Diet; *IMOD*, Italian Mediterranean Organic Diet; *LDL*, low-density lipoprotein; *LPD*, low protein diet; *LPS*, lipopolysaccharide; *MDiet*, Mediterranean diet; *MetS*, metabolic syndrome; *MHMD*, moderately hypocaloric Mediterranean diet; *MTHFR*, methylenetetrahydrofolate reductase; *MUFA*, monounsaturated fatty acids; *N*, total number of subjects; *SFA*, saturated fatty acid; *TAC*, total antioxidant capacity.
Source: Adapted from Di Daniele et al. (2017).

2 Unveiling the potential of probiotics

Russian-born Ilya Ilich Mechnikov (1845–1916) proposed the concept of probiotics in 1908. He was a zoologist and microbiologist who observed that the ingestion of fermented foods, particularly lactic acid bacteria in yogurt-prompted beneficial effects on human health promoting longevity. He is known to have developed a diet with milk fermented with the bacterium he called "Bulgarian bacillus." Ever since his discovery, various studies have confirmed the positive impact of probiotics on host health (Cremon, Barbaro, Ventura, & Barbara, 2018).

Probiotics are broadly defined as live, nonpathogenic microorganisms that possess beneficial properties that positively influence the health of the host when it is administered in adequate quantities (Hill et al., 2014; Howarth & Wang, 2013). Probiotics are usually bacteria from the *Lactobacillus* and *Bifidobacterium* genera, but others like *Lactococcus*, *Streptococcus*, and *Enterococcus* species are also included. Nonpathogenic strains of *Escherichia coli*, and certain *Bacilli* and yeast strains may also act as probiotics (Green et al., 2020; Yakoob, 2019). Probiotics like *Saccharomyces boulardii* (yeast), and bacteria in the *Lactobacillus* and *Bifidobacterium* are naturally found in the intestines of humans. It is worth noting that probiotics occur naturally in foods aside yogurt and they are also present in fermented foods like cheeses, pickles, and sourdough bread.

Probiotics have proven to possess therapeutic properties, including the potential to improve oral health, minimize the severity of diseases ranging from inflammatory skin diseases like dermatitis, inflammatory bowel diseases (e.g., Crohn's disease and ulcerative colitis), to a variety of other cancers (Czarnecka-Operacz & Sadowska-Przytocka, 2017; Howarth & Wang, 2013). In the body of the host, they may prevent the overgrowth of pathogens and act as barriers or resist the growth of pathogens all together (Cremon et al., 2018; Ettinger, MacDonald, Reid, & Burton, 2014). They can actively compete with pathogens in the host's microbial system and are capable of producing numerous metabolites, enzymes and cofactors, and nutrients that become lively in regulating metabolism and improving health. For instance, some probiotics produce vitamins like K, B_2, B_{12}, and folate and aid in carbohydrate digestion to produce short-chain fatty acids, such as butyrate, propionate that can be utilized for energy and improve health. Probiotics' strength lies in their quantity and ability to survive the maze in the body of the host and remain in a viable state to function. Thus they must sustain their life to act because that is necessary to exert the probiotic effect (Sagheddu, Guidesi, Galletti, & Elli, 2019).

However, it has also been speculated that some of the mechanisms behind the probiotic health effects may not be dependent on the viability of the cells and also nonviable probiotics could have some health benefits (https://www.researchgate.net/publication/256291452_Probiotic_viability_-_does_it_matter). Fig. 14.2 shows health benefits of probiotics.

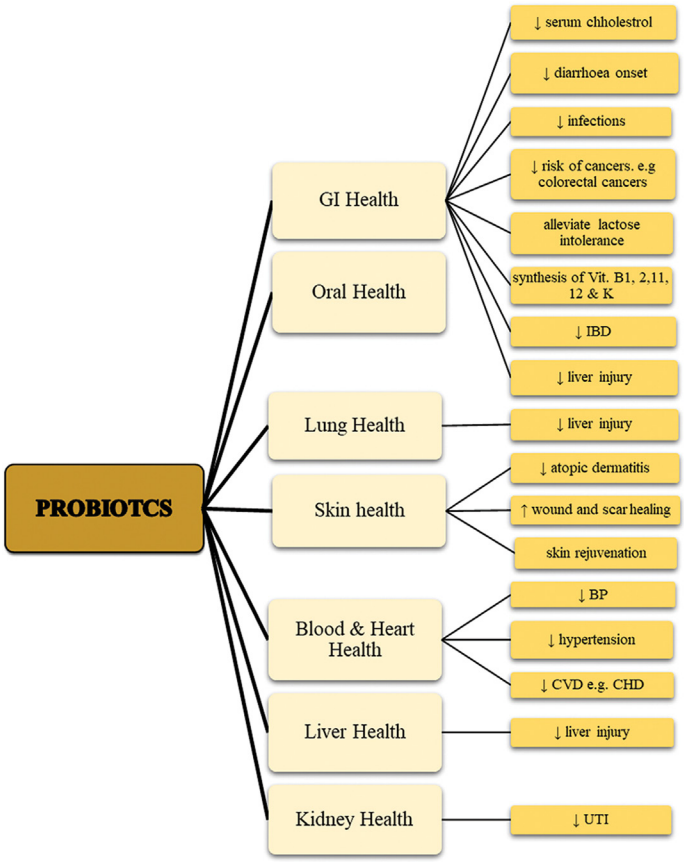


FIGURE 14.2 Health benefits of probiotics. ↑, Increase; ↓, Reduce; BP, blood pressure; CHD, coronary heart disease; CVD, cardiovascular diseases; IBD, inflammatory bowel disease; UTI, urinary tract infection; Vit., vitamin.

TABLE 14.3 Types of dysbiosis.

Bloom of pathobionts	Loss of commensals	Loss of diversity
Pathobionts are members of the commensal microbiota that have pathological tendencies. Even though they are relatively low, they multiply when they are anomalies in the intestinal ecosystem. A typical example is the bloom of *Enterobacteriaceae* observed in patients with inflammatory bowel diseases (IBD) (Levy et al., 2017; Principi, Cozzali, Farinelli, Brusaferro, & Esposito, 2018)	This results from reduced ability of microbiomes to multiply or an exposure that led to the loss of some microbiomes, and it is characterized by the reduction or total loss of normally existing microbiota members (Levy et al., 2017)	An aberration with the occurrence of alpha diversity such that there is a reduction in production. Diet is one of the factors that stimulates increase in the richness of intestinal microbiota which is associated with good metabolic health. Alterations to dietary pattern and health conditions like Type 1 diabetes results in decrease alpha diversity activity—a feature of dysbiosis (Levy et al., 2017)

2.1 Probiotics and dysbiosis

Despite the enormous popularity of probiotics in recent times, there is still circularity with regulations about its optimal usage. There is no clarity yet whether probiotics should be administered as a medical food, drug, or a food supplement. This leaves much to say as concept of probiotics is still a topic under exploration. For the most part, probiotics are sold on the shelves as dietary supplements in the United States. As dietary supplements they are not permitted to claim disease curing therapeutic effects. However, in other parts of the world like the Europe and United Kingdom, probiotics are acknowledged as potent enough to make health claims but not before apt scrutiny and validation. Thus health claims must be adequately backed with evidence from appropriately designed and conducted human clinical studies. As a matter of fact, even in most cases, the food safety authorities rejected most of the health claims submitted to them (McFarland, 2014). However, one of the functional claims made about probiotics is their ability to treat or restore dysbiosis.

Dysbiosis is a health condition characterized by the disruption or imbalance of naturally occurring organisms in the microbiota of a person. It may occur as a result of side effect from using antibiotics or other exposures and thus lead to the overgrowth of opportunistic pathogens that are thought to contribute to a range of health problems especially problems of the gut (McFarland, 2014). It can be referred to as a discrete microbial ecological state that is fundamentally linked to the manifestation or diagnosis of a particular disease. Bloom of pathobionts, loss of commensal, and loss of diversity are mutually nonexclusive characteristics associated with dysbiosis (Table 14.3). The instigating causes of dysbiosis include genetics, infections and inflammation, diet and xenobiotics, familial transmission (i.e., maternal microbiota shapes fetal microbiomes), environmental microbiome transmissions (i.e., changes in one's microbiome as a result of environmental factors), and a host of others (Levy, Kolodziejczyk, Thaiss, & Elinav, 2017).

Even though the mechanism is not fully understood, probiotics have been found to correct or restore dysbiosis by attempting to normalize microbiota after it has been disrupted. When using probiotics to correct dysbiosis, one of the following could occur: a total restoration of the disrupted microbiota, an improvement after the disruption or no substantial change at all. Thus there are three different degrees of probiotic effect (restoration, improvement, or no effect) (McFarland, 2014). Moreover, the link between clinical efficacy and a probiotic's ability to restore the microbiota is a crucial determinant for understanding the mechanism for the action of probiotics. For example, a probiotic that possesses a greater ability to restore the microbiota is associated with the highest strength of clinical efficacy. So far, the factors that seem to pose challenges with making appropriate recommendations of probiotics have to do with, the diversity of probiotic products available, inconclusive proof of effectiveness uncertainty of the probiotics in clinical trials, and most importantly the absence of standard that defines what constitutes a "normal" microbiota. The latter is important because it is a yard stick to fully establish a cause and effect between dysbiosis and the role of probiotics (Ettinger et al., 2014; McFarland, 2014). Undoubtedly, more studies are required to make concrete recommendations on the type, efficacy, and quantity of probiotics in relation to normal microbiota health and the correction of dysbiosis. This would need to clarify and strengthen the requirements for evidence to substantiate health claims.

3 Gut microbiota in obesity

The role of gut microbiota (GM) in host physiology and metabolism include glucose and lipid homeostasis, immunity, intestinal barrier integrity, inflammation, and other key metabolic processes that has been well documented over the years (Dave, Higgins, Middha, & Rioux, 2012; DuPont & DuPont, 2011; Ley, Turnbaugh, Klein, & Gordon, 2006).

Current knowledge has established the pathogenesis of several diseases which arises from perturbation of the structure and/or function of the GM. Several factors, including diet, age, surgery, stress, BMI, and pregnancy and antibiotics, are known to influence the composition and/or functionality of the GM (Costello, Stagaman, Dethlefsen, Bohannan, & Relman, 2012; Dave et al., 2012; DuPont & DuPont, 2011). Increased abundance of Firmicutes and decreased level of Bacteroidetes have been proposed as a marker in obesity. Several studies have confirmed a higher ratio of Firmicutes to Bacteroidetes (F/B), two major bacterial phyla in the GM in obese individuals (Kasai et al., 2015; Koliada et al., 2017; Ley et al., 2006; Turnbaugh et al., 2009, 2006). Nonetheless, other studies have shown no correlation regarding the F/B ratio and obesity and reported no significant differences between these two phyla (Haro et al., 2016; Hu et al., 2015; Koliada et al., 2017). Therefore the use of F/B ratio to predict obesity still remains controversial and requires further investigations to understand the complex bidirectional relationship between GM and obesity, and the interaction between host physiology and gut flora.

3.1 Probiotics and obesity

Obesity is the excess accumulation of fat resulting from imbalance between energy intake and expenditure over long periods of time. The etiology of obesity is known to be caused by a complex interaction of genetic and environmental factors (Hill, 2006). Among these, dietary habits (consumption of foods high in fat or carbohydrates) and sedentary lifestyles contribute significantly to the rising trend of obesity globally (https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death, 2016). Obesity increases the incidence of MetS related with other conditions such as hypercholesterolemia, hyperglycemia, and hypertension and is associated with diabetes, CVD, and dementia as already discussed (Fan et al., 2017; Kent et al., 2019). However, the difficulty of maintaining weight loss through lifestyle changes (healthy diet and active lifestyle) has led to finding other strategies to prevent weight gain and hence the development of MetS (Anderson, Konz, Frederich, & Wood, 2001).

Recent evidence has revealed obesity as both a cause and consequence of GM dysbiosis (Bray, Frühbeck, Ryan, & Wilding, 2016). As a major contributor to obesity, the GM has been shown to affect host energy harvest and storage (Bäckhed et al., 2004; Cox, West, & Cripps, 2015). Several studies have reported an association of obesity with a decrease in bacterial richness and diversity (Gao et al., 2018; Liu et al., 2017). Notwithstanding, the safety and benefits of probiotics on human health such as metabolism and immunity have gained increasing attention over the years (Khalesi et al., 2019; Koutnikova et al., 2019). Available evidence suggests GM modulation with probiotics supplementation by decreasing gut permeability, inflammation, and metabolic disorders. Thus the GM has become an attractive target due to its association with the pathogenesis of obesity and other metabolic disorders; therefore their restoration or manipulation with dietary probiotic supplementation is being explored as an effective approach for the prevention of obesity and MetS (Sun et al., 2018).

The role of probiotics as valuable tools in the prevention or treatment of chronic low-grade inflammation that characterizes MetS has been discussed elsewhere in a review (Torres, Fabersani, Marquez, & Gauffin-Cano, 2019). Despite the health promoting (antiobesogenic) effects of probiotics, some studies have reported no beneficial or even obesogenic effects associated with probiotics. Findings from several systematic and metaanalysis that evaluated the effects of probiotic consumption on obesity and metabolic outcomes have shown variation and inconsistencies. A systematic review and meta-analysis of randomized controlled trials (RCTs) showed that short-term ($\leq$12 weeks) probiotic supplementation reduced body weight, BMI, and fat percentage compared with placebo, but the effect sizes were small. Moreover, the administration of probiotics did not show any significant effect on fat mass (Borgeraas, Johnson, Skattebu, Hertel, & Hjelmesaeth, 2018). Another metaanalysis involving 23 RCTs revealed that synbiotic supplementation could decrease body weight and waist circumference. However, no significant effect on BMI and body fat was found in comparison with placebo group. In addition, dosage of probiotics did not show any effect on anthropometric measurements. Nonetheless, synbiotic supplementation had a moderate effect on body weight and waist circumference through the manipulation of GM composition (Hadi, Alizadeh, Hajianfar, Mohammadi, & Miraghajani, 2020). Similarly, a systematic review and metaanalysis conducted with 19 randomized trials, including 1412 participants, revealed only a moderate reduction on waist circumference with oral supplementation of probiotics or synbiotics; however, no effect on body weight and BMI was found despite a low-to-moderate quality of evidence (Suzumura et al., 2019).

A metaanalysis from 12 human studies that examined the effects of probiotic supplementation on blood lipid levels [total cholesterol (TC), LDL, HDL, and TG] in obese or overweight people found a significant decrease in TC and LDL in the probiotic group compared with the control. However, HDL and TG concentrations between both groups were not significantly different. Therefore larger trials needed to confirm the efficacy and safety of probiotics in the regulation of blood lipid levels in obese or overweight people (Yan, Tian, Li, Li, & Cui, 2019). In contrast a systematic review on probiotic supplementation

for the management of obesity involving 72 animal studies and 15 clinical trials revealed the beneficial effects of probiotics on body weight parameters via the modulation of GM. However, these positive outcomes were observed to be species and strain specific (Ejtahed et al., 2019). Similarly, the findings from a metaanalysis conducted by Tenorio-Jiménez, Martínez-Ramírez, Gil, & Gómez-Llorente, (2020) on the effect of probiotics on MetS components in RCTs showed that the intake of probiotics in MetS patients resulted in improved BMI, glucose metabolism, blood pressure, lipid profile and also had positive impact on inflammatory biomarkers such as tumor necrosis factor-α and interleukine-6 in some studies. Taken together, probiotic intake could provide some benefits in alleviating MetS conditions or characteristics although effects are marginal compared to drug therapy. However, it is worthy to note that the specific strains, dosage, and duration of intervention may play a significant role in the prevention and treatment of MetS outcomes Tenorio-Jiménez, et al., (2020).

Moreover, new evidence suggests the importance of baseline GM characteristics in obese patients in determining the efficacy of nutritional intervention specifically with prebiotics. The presence of preintervention levels of specific bacteria consortia is known to positively influence the metabolic outcomes related to obesity Rodriguez et al., (2020). However, as to whether weight loss in humans during microbiome-based probiotic interventions could be predicted by baseline GM or aspects of host physiology are still not clearly understood.

According to Mullish et al. (2021), rather than gut microbial signatures, weight loss in microbial intervention (probiotic) studies may be mediated by changes in abnormal host metabolic functions due to the complexity of the disease with varying dominant mechanisms in different individuals. Moreover, whether fecal metabolites such as sulfated bile acids are direct or indirect indicators influencing weight loss remains to be fully unraveled (Mullish et al., 2021).

3.1.1 Animal studies

The supplementation of *Hafnia alvei* HA4597, a food grade commensal bacteria expressing the ClpB protein with an α-melanocyte stimulating hormone-like motif was found to decrease appetite, body weight, and fat mass gain in hyperphagic and obese mice. Findings from this study revealed the anorexigenic and lipolytic effects in hyperphagic ob/ob mice resulting in decreased fat mass and body weight gain. The occurrence of low enterobacterial ClpB gene abundance in the microbiota of obese humans validates *H. alvei* HA4597 as a new potential probiotic for appetite and body weight control in overweight and obesity (Legrand et al., 2020). In another study, this research group demonstrated the probiotic efficacy of *H. alvei* HA4597 in exerting antiobesity and metabolic effects in obese male mice characterized by hyperphagia and diet-induced adiposity. High-fat-diet (HFD)-fed obese mice treated daily with 1.4×10^{10} colony-forming unit (CFU) of *H. alvei* for 38 days showed a decrease in body weight and food intake similar to normal control group (mice fed standard diet). Furthermore, *H. alvei*-treated mice also showed a decrease in glycemia, plasma TC, and alanine aminotransferase compared with the orlistat-treated group (Lucas et al., 2019).

Kong, Gao, Yan, Huang, and Qin (2019) found that probiotic supplementation of HFDs and high-sucrose diets (HCDs)-induced obese mice delayed weight gain partly by modulating GM especially in HCD groups. Probiotic preparation containing equal ratio of *Lactobacillus acidophilus*, *Bifidobacterium longum*, and *Enterococcus faecalis* was administered daily at a dose of 2.0×10^7 CFU for 30 days. In addition, microbial diversity and abundance of beneficial bacteria such as *Lactobacillus* and *Prevotella* was reduced in both HFD and HCD groups. However, pathogenic bacteria, including *Bacteroides*, *Alistipes*, and *Anaerotruncus*, were observed to increase. Restoration and an increase in abundance of microbiota negatively correlated with obesity such as *Lactococcus*, *Bifidobacterium*, and *Akkermansia* in probiotic-fed groups (Kong et al., 2019). Similarly, HFD-induced obese mice orally supplemented with *Bacillus licheniformis* were found to have a reduced body weight, fat accumulation, and an improved glucose tolerance by modulating colonic microflora (Cao et al., 2019).

Le Barz et al. (2019) screened the immunomodulatory effects of bacterial strains isolated from dairy products and human feces in vitro. The authors further evaluated the potential of three *Lactobacillus* strains (*Lactobacillus plantarum*, Lb38; *Lactobacillus paracasei*, L79; and *Lactobacillus rhamnosus*, Lb102) and two *Bifidobacterium* strains (*Bifidobacterium animalis* ssp. *lactis* species, Bf26 and Bf141) administered for 2 months at a dose of 10^9 in a high-fat high-sucrose diet-induced obese C57BL/6 mice to improve metabolic outcomes. Probiotic strains Lb102 and Bf141 reduced visceral obesity, inflammation and improved glucose tolerance and insulin sensitivity. Furthermore, intestinal integrity markers were enhanced in Lb102- and Bf141-fed groups with associated changes in microbiota composition (Le Barz et al., 2019). Another study that investigated the effect of supplementation of *Eurotium cristatum*, a dominant fungus in Fuzhuan brick tea preparation, and storage in HFD-induced obese C57BL/6J mice revealed that *E. cristatum* consumption prevented body weight gain and fat accumulation by modulating the bacterial and fungal composition, therefore partially reversing dysbiosis. Acetate and butyrate-producing bacteria increased in *E. cristatum*-fed mice compared to untreated ones with higher levels of butyrate in fecal samples. These findings suggest that *E. cristatum* with beneficial effects to alleviate obesity could be used as a potential probiotic fungus (Kang, Su, Duan, & Huang, 2019).

New evidence suggests the importance of baseline GM characteristics in obese patients in determining the efficacy of nutritional intervention. In a recent study, Rodriguez et al. (2020) compared the metabolic and microbiota changes in humanized obese mice (*hum-ob* mice; colonized with fecal samples from different obese donors) with obese individuals supplemented with the prebiotic inulin for 3 months. *Hum-ob* mice responded differently to inulin supplementation. The presence of specific bacteria consortia, including *Barnesiella*, *Bilophila*, *Butyricimonas*, *Victivallis*, *Clostridium* XIVa, *Akkermansia*, *Raoultella*, and *Blautia*, rather than bacterial diversity correlated with improved metabolic disorders such as decrease in adiposity and hepatic steatosis in *hum-ob* mice. In addition, the decrease of body mass index in obese individuals was found to be dependent on the preintervention levels of *Anaerostipes*, *Akkermansia*, and *Butyricicoccus*. Therefore these bacteria consortia could be considered as new targets in the management of metabolic disorders linked to obesity.

The modulation of host gene expression and metabolism by probiotics and their beneficial effect on complex organs like the adipose tissue development and function has been suggested by recent evidence (Cerdó, García-Santos, Bermúdez M., & Campoy, 2019). The effect of exopolysaccharide-producing *B. animalis* IPLA R1 on short-term diet-induced obese C57BL/6J mice's glucose, lipid metabolism, and gut microbial composition was investigated by Salazar et al. (2019). *B. animalis* IPLA R1-supplemented mice showed a significant decrease in TG and fasting serum insulin accumulation. The expression of genes involved in the hepatic synthesis of fatty acids were downregulated, while those related with fatty acid oxidation were upregulated in *B. animalis* IPLA R1 fed mice. Furthermore, an increase in *Bifidobacterium* and *Bacteroides–Prevotella* levels was observed in *B. animalis* IPLA R1-fed groups compared to the untreated group (Salazar et al., 2019). Probiotic supplementation in animal models has shown very promising results; however, outcomes from most human clinical trials need larger and well-designed studies to confirm their efficacy of use. In addition, the emergence of next-generation probiotic bacterial strains with beneficial effects for human health such as *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, and *Clostridium* clusters IV, XIVa, and XVIII, together with advances in sequencing tools, bioinformatics, metabolomics, and personalized nutrition or medicine would increase the future prospective of overcoming present challenges.

3.1.2 Clinical trials

Several human clinical studies of the effect of probiotic supplementation in overweight/obese subjects are shown in Table 14.4. In a large cross-sectional study involving 38,802 adults, 13.1% reported consuming yogurt or a probiotic supplement during the 24-h dietary recall or dietary supplement use 30-day questionnaire. BMI was significantly lower and the prevalence of obesity was lower in the probiotic group (Lau, Neves, Ferreira-Magalhães, Carvalho, & Freitas, 2019). Sergeev, Aljutaily, Walton, and Huarte (2020) conducted an intervention study that examined the effects

TABLE 14.4 Human clinical trials of probiotic supplementation on overweight/obesity outcomes.

Study design	Sample	Duration of intervention	Purpose of the study	Clinical outcomes	References
Randomized, double-blind controlled	220 Healthy overweight/obese Bulgarian adults (30–65 years)	6 Months	Assessed the effects of probiotic consortium Lab4P (*Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Bifidobacterium bifidum*, and *Bifidobacterium animalis* subsp. *lactis*) supplementation at 50 billion CFU/day on body weight and well-being	Significant decrease in body weight, BMI, WC, and WtHR in probiotic group. Females recorded greater weight loss. Improvement in sdLDL-C profiles, QoL, and URTI incidence	Michael et al. (2020)
Randomized, double-blind controlled	130 Overweight/obese pregnant Iranian women with no diabetes (29–30 years)	4 Months	Examined the effects of probiotic yoghurts (*L. acidophilus* La5 and *B. lactis* Bb12) supplementation on maternal plasma glucose and infant complications	Significantly lower blood plasma levels in probiotic group. Infants from probiotic group mothers had significantly lower bilirubin level 3–5 days after birth	Asgharian, Homayouni-Rad, Mirghafourvand, and Mohammad-Alizadeh-Charandabi (2020)

(*Continued*)

TABLE 14.4 Human clinical trials of probiotic supplementation on overweight/obesity outcomes. (*Cont.*)

Study design	Sample	Duration of intervention	Purpose of the study	Clinical outcomes	References
Randomized, double-blind controlled	49 Healthy obese adults (53 ± 8 years)	3 Months	The aim was to evaluate the effect of 3 different probiotic strains (*Bacillus subtilis* R0179, *L. plantarum* HA-119, and *B. animalis* subsp. *lactis* B94) on plasma and stool bile acids, lipids, glucose, and insulin levels	Increased deconjugated plasma bile acids in *B. subtilis* and *B. lactis* group compared to placebo. Probiotics had no effect on glucose metabolism or serum cholesterol	Culpepper et al. (2019)
Randomized, double-blind controlled	126 Obese individuals (>18 years)	3 Months	To assess the effect of *B. animalis* subsp. *lactis* CECT 8145 and its heat-killed form on anthropometric adiposity biomarkers	Significant decrease in BMI, WC, WC/H ratio, visceral fat in probiotic treated compared to placebo group and specifically in women. Inverse relationship between increase in *Akkermansia* spp. and weight in probiotic group	Pedret et al. (2019)
Randomized double-blinded placebo-controlled	50 Obese pregnant women (30 ± 5 years)	6 Months	To investigate the effects of multistrain probiotic intervention on GWG, GDM, maternal glucose homeostasis, infant birth weight, and maternal gut microbiota	No significant difference in HbA1c levels, occurrence of GDM, GWG, and infant birth weight between groups Increase in microbial diversity in probiotic group	Halkjær et al. (2020)
Metabolic-Nutritional-Psychological Rehabilitation Program	20 Elderly obese women (≥65 years)	1 Month	To assess the effect of short-term hypocaloric Mediterranean diet and probiotic administration on gut microbiota modulation and metabolic markers	Mediterranean diet decreased weight and partially reversed dysbiosis. Higher weight loss, improved oxidative stress markers, and increased abundance of *Akkermansia* after Mediterranean diet supplemented with probiotics	Cancello et al. (2019)
Randomized double-blinded placebo-controlled	50 Obese women (45–70 years)	3 Months	To evaluate the effects of multispecies probiotic (contain 3 *Bifidobacteria* strains and 6 *Lactobacilli* strains) supplementation on homocysteine (Hcy) levels, oxidative stress, inflammation, and lipid profile	Significant decrease in Hcy, TNF-α, TC, LDL, and TG in probiotic group. Oxidative status was improved in probiotic group	Majewska, Kręgielska-Narożna, Jakubowski, Szulinska, and Bogdanski (2020)
Randomized double-blinded placebo-controlled	32 Overweight or obese women	3 Months	Assessed the effects of probiotic mixture containing 3 *Lactobacillus* and 2 *Bifidobacteria* strains on metabolic and gut microbiota modulation	*Clostridiaceae* was negatively associated with TNF-α. Higher proportion of TM7 division was correlated with higher adiposity. Increase abundance of *Clostridiaceae* and decrease in candidate TM7 in probiotic group	Gomes, Hoffmann, and Mota (2020)

of a synbiotic supplement (comprising four probiotics, *L. acidophilus*, *B. lactis*, *B. longum*, and *Bifidobacterium bifidum* and galactooligosaccharide as the prebiotic component) on 20 obese human subjects metabolic biomarkers and GM composition and richness participating in weight loss program based on a low-carbohydrate, high-protein, reduced energy diet. At the end of the 3-month intervention, no significant difference was observed in body mass, BMI, body fat mass, body fat percentage, and body lean mass between the synbiotic and placebo groups. An association between an increase in *Lactobacillus* abundance and a decrease in blood glucose was observed. Also, an increase in abundance and richness of *Bifidobacterium* and *Lactobacillus* was found in symbiotic supplemented groups. However, synbiotic treatment did not show significant impact on weight loss parameters but modulated the GM that could help one to reduce detrimental effects of high protein diet (Sergeev et al., 2020).

DNA methylation has been implicated in several biological processes and diseases via its alteration and impact on gene activity (Kirchner, Osler, Krook, & Zierath, 2013). Diet is reported to modify this process that results in health improvement. The effect of probiotic supplementation on modifications of DNA methylation status of obesity and weight gain–related promoter genes in mothers and their children was assessed in a pilot study involving 15 pregnant women. In the probiotic group, DNA methylation levels in 37 and 68 gene promoters were significantly decreased in mothers and children, respectively. However, an increase in DNA methylation was observed in one gene promoter in mothers (Vähämiko et al., 2019).

4 Probiotics and cardiovascular diseases

According to the WHO and the American Heart Association, CVDs result from any category of chronic disorders related to the heart and blood vessels. They include diseases resulting from defects of the heart's structure and its supporting branches such as ventricles, veins, valves, and arteries. CVDs are mostly caused from slowing or obstruction of blood flow, that is, defects in main arteries that supply flow of oxygen and nutrients to the body and clots that limit oxygen to the brain, resulting in strokes often affecting other parts of the body such as kidneys, lungs and legs; and atherosclerosis, the narrowing and hardening of blood vessels that result in heart attacks, stroke, and aneurysm. CVDs include coronary heart disease (CHD), cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease, deep vein thrombosis, and pulmonary embolism.

CVD tops the chart of the leading causes of death worldwide and in the United States (https://www.nhlbi.nih.gov/health-topics/coronary-heart-disease, 2019) (https://www.cdc.gov/heartdisease/facts.htm, 2020). WHO reported that globally, 15.2 million deaths in 2016 were attributed to ischemic heart disease and stroke and they have stayed the leading cause of death for the last 15 years. Ischemic heart disease is a kind of CHD. CHD is caused by a narrowing of the coronary arteries that supply the heart and is known to be the leading form of CVD with more than 500,000 Americans dying of heart attacks caused by CHD annually. It is true that certain nonmodifiable factors such as age, gender, and genetics are risk factors for CVD. However, research purports that many of the deaths could be prevented because CHD is related to modifiable risk factors like lifestyle, smoking, diabetes (Type 2, mainly due to weight, lifestyle choices), stress, excessive alcohol intake, hypertension, and physical inactivity and elevated blood lipids (cholesterol, TGs) increase the risk of getting CVDs. Consequently, nutrition has proven to positively influence a bulk of CVD risk factors. Reducing sodium levels by reducing salt intake, eating nutrient diverse foods as in, for example, the Dietary Approaches to Stop Hypertension (DASH) diet, including fruits, vegetables, fibrous foods, and monounsaturated and omega-3 fatty acids in diets are all ways nutrition has shown to reduce the risk factors of CVDs.

There is emerging evidence that probiotics can contribute marginally to improve and maintain cardio health. Primarily, the action of probiotics can be seen by reducing risk factors like hypertension and high cholesterol levels that can be associated with CVDs. In the case of reducing lipid levels, probiotics promote liver health. They do the former by producing acids that is, propanoic acid that controls the production of cholesterol by the liver. The liver is responsible for bile production (bile is composed of water, electrolytes, and organic molecules, including bile acids, phospholipids, and bilirubin), which is needed for the emulsification of lipids. The liver produces bile acids (i.e., a component of bile) from cholesterol. Probiotics have disintegrating effect on bile acids (i.e., increase bile acid excretion) such that the liver would need to expend more cholesterol in producing more bile acids and ultimately bile. Probiotics literally feed on cholesterol and their action reduces the lipid levels greatly (Saini, Saini, & Sharma, 2010).

Several hypotheses on cholesterol reduction by probiotic bacteria have been proposed. These include the binding of cholesterol to the bacterial cells, cholesterol precipitation by bile acids, metabolizing by hydrolase activity and deconjugation of bile acids by bile salt hydrolase (BSH). However, the latter is currently the most plausible action mechanism. Lee et al.'s groups have extensively studied on genetics and structure of BSH (Dong & Lee, 2018; Liong et al., 2015; Ishimwe, Daliri, Lee, Fang, & Du, 2015; Kim & Lee, 2008). Another way of probiotics lowering cholesterol is by binding to cholesterol molecules in the gut, which are often excreted. Thus preventing the absorption of cholesterol into the blood altogether.

Having already mentioned, the efficacy of probiotics is dependent on the type of species and strain used. There is emerging evidence that probiotics can provide direct cardioprotective effect to the heart that results in improved cardiac health; however, more evidence-based human trials are required to fully assert the mechanism of action of probiotics for combating CVDs (Ebel et al., 2014; Ettinger et al., 2014; Saini et al., 2010).

5 Conclusion

The global rise in the prevalence and incidence of MetS is now a chronic plague of the 21st century. Besides genetic and environmental factors, lifestyle such as sedentary habits, reduced physical activity, and poor dietary habits have been found to significantly contribute to the increase risk of MetS. Current evidence shows the pathogenesis of several diseases, including MetS resulting from the perturbation of the structure and/or function of the GM. Hence, the genetic modification has become an attractive target due to its involvement and role in host physiology and metabolism in the prevention and management of MetS. The benefits of probiotics or probiotic-containing foods on human health have gained increasing attention over the years. However, it is worthy to note that the efficacy of probiotics is dependent on the type (specific) strains, dosage, and duration of intervention. Moreover, the importance of baseline GM characteristics and changes in host physiology and metabolic function in MetS patients in determining the efficacy of nutritional and or probiotic intervention should be taken into consideration. Furthermore, the emergence of next-generation probiotic bacterial strains with beneficial effects for human health such as *A. muciniphila*, *F. prausnitzii*, and *Clostridium* clusters IV, XIVa, and XVIII, together with advances in sequencing tools, bioinformatics, metabolomics, and personalized nutrition or medicine would increase the future prospective of overcoming present challenges. Finally, well-designed larger clinical trials are still needed to confirm the efficacy of probiotic use.

References

Anderson, J. W., Konz, E. C., Frederich, R. C., & Wood, C. L. (2001). Long-term weight-loss maintenance: A meta-analysis of US studies. *The American Journal of Clinical Nutrition*, *74*(5), 579–584. doi: 10.1093/ajcn/74.5.579.

Asgharian, H., Homayouni-Rad, A., Mirghafourvand, M., & Mohammad-Alizadeh-Charandabi, S. (2020). Effect of probiotic yoghurt on plasma glucose in overweight and obese pregnant women: A randomized controlled clinical trial. *European Journal of Nutrition*, *59*(1), 205–215. doi: 10.1007/s00394-019-01900-1.

Bäckhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., et al. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(44), 15718–15723. doi: 10.1073/pnas.0407076101.

Bassi, N., Karagodin, I., Wang, S., Vassallo, P., Priyanath, A., Massaro, E., et al. (2014). Lifestyle modification for metabolic syndrome: A systematic review. *The American Journal of Medicine*, *127*(12), 1242.e1-e10. doi: 10.1016/j.amjmed.2014.06.035.

Basu, A., Du, M., Leyva, M. J., Sanchez, K., Betts, N. M., Wu, M., et al. (2010a). Blueberries decrease cardiovascular risk factors in obese men and women with metabolic syndrome. *The Journal of Nutrition*, *140*(9), 1582–1587.

Basu, A., Fu, D. X., Wilkinson, M., Simmons, B., Wu, M., Betts, N. M., et al. (2010b). Strawberries decrease atherosclerotic markers in subjects with metabolic syndrome. *Nutrition Research*, *30*(7), 462–469.

Borgeraas, H., Johnson, L. K., Skattebu, J., Hertel, J. K., & Hjelmesaeth, J. (2018). Effects of probiotics on body weight, body mass index, fat mass and fat percentage in subjects with overweight or obesity: A systematic review and meta-analysis of randomized controlled trials. *Obesity Reviews*, *19*(2), 219–232. doi: 10.1111/obr.12626.

Bray, G. A., Frühbeck, G., Ryan, D. H., & Wilding, J. P. H. (2016). Management of obesity. *The Lancet*, *387*(10031), 1947–1956. https://doi.org/10.1016/S0140-6736(16)00271-3.

Cancello, R., Turroni, S., Rampelli, S., Cattaldo, S., Candela, M., Cattani, L., et al. (2019). Effect of short-term dietary intervention and probiotic mix supplementation on the gut microbiota of elderly obese women. *Nutrients*, *11*(12), 3011. doi: 10.3390/nu11123011.

Cao, G., Dai, B., Wang, K., Yan, Y., Xu, Y., Wang, Y., et al. (2019). *Bacillus licheniformis*, a potential probiotic, inhibits obesity by modulating colonic microflora in C57BL/6J mice model. *Journal of Applied Microbiology*, *127*(3), 880–888.

Cerdó, T., García-Santos, J. A., Bermúdez M., G., & Campoy, C. (2019). The role of probiotics and prebiotics in the prevention and treatment of obesity. *Nutrients*, *11*(3), 635.

Costello, E. K., Stagaman, K., Dethlefsen, L., Bohannan, B. J., & Relman, D. A. (2012). The application of ecological theory toward an understanding of the human microbiome. *Science*, *336*(6086), 1255–1262.

Cox, A. J., West, N. P., & Cripps, A. W. (2015). Obesity, inflammation, and the gut microbiota. *The Lancet Diabetes & Endocrinology*, *3*(3), 207–215. https://doi.org/10.1016/S2213-8587(14)70134-2.

Cremon, C., Barbaro, M. R., Ventura, M., & Barbara, G. (2018). Pre-and probiotic overview. *Current Opinion in Pharmacology*, *43*, 87–92.

Cuenca-García, M., Artero, E. G., Sui, X., Lee, D. -C., Hebert, J. R., & Blair, S. N. (2014). Dietary indices, cardiovascular risk factors and mortality in middle-aged adults: Findings from the Aerobics Center Longitudinal Study. *Annals of Epidemiology*, *24*(4), 297–303. e292.

Culpepper, T., Rowe, C. C., Rusch, C. T., Burns, A. M., Federico, A. P., Girard, S. A., et al. (2019). Three probiotic strains exert different effects on plasma bile acid profiles in healthy obese adults: Randomised, double-blind placebo-controlled crossover study. *Beneficial Microbes*, *10*(5), 497–509. doi: 10.3920/bm2018.0151.

Czarnecka-Operacz, M., & Sadowska-Przytocka, A. (2017). Probiotics for the prevention of atopic dermatitis and other allergic diseases: What are the real facts? *Alergologia Polska-Polish Journal of Allergology*, *4*(3), 89–92.

Dave, M., Higgins, P. D., Middha, S., & Rioux, K. P. (2012). The human gut microbiome: Current knowledge, challenges, and future directions. *Translational Research*, *160*(4), 246–257.

De Lorenzo, A., Noce, A., Bigioni, M., Calabrese, V., Della Rocca, D., Daniele, N., et al. (2010). The effects of Italian Mediterranean organic diet (IMOD) on health status. *Current Pharmaceutical Design*, *16*(7), 814–824.

De Lorenzo, A., Petroni, M., De Luca, P. P., Andreoli, A., Morini, P., Iacopino, L., et al. (2001). Use of quality control indices in moderately hypocaloric Mediterranean diet for treatment of obesity. *Diabetes Nutrition and Metabolism*, *14*(4), 181–188.

Di Daniele, N., Noce, A., Vidiri, M. F., Moriconi, E., Marrone, G., Annicchiarico-Petruzzelli, M., et al. (2017). Impact of Mediterranean diet on metabolic syndrome, cancer and longevity. *Oncotarget*, *8*(5), 8947.

Di Daniele, N., Petramala, L., Di Renzo, L., Sarlo, F., Della Rocca, D. G., Rizzo, M., et al. (2013). Body composition changes and cardiometabolic benefits of a balanced Italian Mediterranean diet in obese patients with metabolic syndrome. *Acta Diabetologica*, *50*(3), 409–416.

Dong, Z., & Lee, B. H. (2018). Bile salt hydrolases: Structure and function, substrate preference, and inhibitor development. *Protein Science*, *27*(10), 1742–1754.

DuPont, A. W., & DuPont, H. L. (2011). The intestinal microbiota and chronic disorders of the gut. *Nature Reviews Gastroenterology & Hepatology*, *8*(9), 523–531.

Ebel, B., Lemetais, G., Beney, L., Cachon, R., Sokol, H., Langella, P., et al. (2014). Impact of probiotics on risk factors for cardiovascular diseases. A review. *Critical Reviews in Food Science and Nutrition*, *54*(2), 175–189.

Ejtahed, H.-S., Angoorani, P., Soroush, A.-R., Atlasi, R., Hasani-Ranjbar, S., Mortazavian, A. M., et al. (2019). Probiotics supplementation for the obesity management; a systematic review of animal studies and clinical trials. *Journal of Functional Foods*, *52*, 228–242. https://doi.org/10.1016/j.jff.2018.10.039.

Ettinger, G., MacDonald, K., Reid, G., & Burton, J. P. (2014). The influence of the human microbiome and probiotics on cardiovascular health. *Gut Microbes*, *5*(6), 719–728. doi: 10.4161/19490976.2014.983775.

Fan, Y. C., Chou, C. C., You, S. L., Sun, C. A., Chen, C. J., & Bai, C. H. (2017). Impact of worsened metabolic syndrome on the risk of dementia: A nationwide cohort study. *Journal of the American Heart Association*, *6*(9), e004749. doi: 10.1161/JAHA.116.004749.

Fenwick, P. H., Jeejeebhoy, K., Dhaliwal, R., Royall, D., Brauer, P., Tremblay, A., et al. (2019). Lifestyle genomics and the metabolic syndrome: A review of genetic variants that influence response to diet and exercise interventions. *Critical Reviews in Food Science and Nutrition*, *59*(13), 2028–2039. doi: 10.1080/10408398.2018.1437022.

Fitó, M., Guxens, M., Corella, D., Sáez, G., Estruch, R., de la Torre, R., et al. (2007). Effect of a traditional Mediterranean diet on lipoprotein oxidation: A randomized controlled trial. *Archives of Internal Medicine*, *167*(11), 1195–1203.

Gao, R., Zhu, C., Li, H., Yin, M., Pan, C., Huang, L., et al. (2018). Dysbiosis signatures of gut microbiota along the sequence from healthy, young patients to those with overweight and obesity. *Obesity (Silver Spring)*, *26*(2), 351–361. doi: 10.1002/oby.22088.

Gomes, A. C., Hoffmann, C., & Mota, J. F. (2020). Gut microbiota is associated with adiposity markers and probiotics may impact specific genera. *European Journal of Nutrition*, *59*(4), 1751–1762. doi: 10.1007/s00394-019-02034-0.

Green, M., Arora, K., & Prakash, S. (2020). Microbial medicine: Prebiotic and probiotic functional foods to target obesity and metabolic syndrome. *International Journal of Molecular Sciences*, *21*(8), 2890. doi: 10.3390/ijms21082890.

Grundy, S. M. (2020). Metabolic syndrome. In E. Bonora, & R. A. DeFronzo (Eds.), *Diabetes complications, comorbidities and related disorders* (pp. 71–107). Cham: Springer International Publishing.

Hadi, A., Alizadeh, K., Hajianfar, H., Mohammadi, H., & Miraghajani, M. (2020). Efficacy of synbiotic supplementation in obesity treatment: A systematic review and meta-analysis of clinical trials. *Critical Reviews in Food Science and Nutrition*, *60*(4), 584–596. doi: 10.1080/10408398.2018.1545218.

Halkjær, S. I., de Knegt, V. E., Lo, B., Nilas, L., Cortes, D., Pedersen, A. E., et al. (2020). Multistrain probiotic increases the gut microbiota diversity in obese pregnant women: Results from a randomized, double-blind placebo-controlled study. *Current Developments in Nutrition*, *4*(7), nzaa095. doi: 10.1093/cdn/nzaa095.

Haro, C., Rangel-Zúñiga, O. A., Alcalá-Díaz, J. F., Gómez-Delgado, F., Pérez-Martínez, P., Delgado-Lista, J., et al. (2016). Intestinal microbiota is influenced by gender and body mass index. *PLoS One*, *11*(5), e0154090.

He, M., & Shi, B. (2017). Gut microbiota as a potential target of metabolic syndrome: The role of probiotics and prebiotics. *Cell & Bioscience*, *7*(1), 54. doi: 10.1186/s13578-017-0183-1.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., et al. (2014). The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*, *11*(8), 506–514. doi: 10.1038/nrgastro.2014.66.

Hill, J. O. (2006). Understanding and addressing the epidemic of obesity: An energy balance perspective. *Endocrine Reviews*, *27*(7), 750–761. doi: 10.1210/er.2006-0032.

Howarth, G. S., & Wang, H. (2013). Role of endogenous microbiota, probiotics and their biological products in human health. *Nutrients*, *5*(1), 58–81. doi: 10.3390/nu5010058.

Hu, H.-J., Park, S.-G., Jang, H. B., Choi, M.-G., Park, K.-H., Kang, J. H., et al. (2015). Obesity alters the microbial community profile in Korean adolescents. *PLoS One*, *10*(7), e0134333.

Ishimwe, N., Daliri, E. B., Lee, B. H., Fang, F., & Du, G. C. (2015). The perspective on cholesterol lowering mechanisms of probiotics. *Molecular Nutrition & Food Research Journal*, *59*, 94–105.

Kang, D., Su, M., Duan, Y., & Huang, Y. (2019). *Eurotium cristatum*, a potential probiotic fungus from Fuzhuan brick tea, alleviated obesity in mice by modulating gut microbiota. *Food & Function*, *10*(8), 5032–5045.

Kasai, C., Sugimoto, K., Moritani, I., Tanaka, J., Oya, Y., Inoue, H., et al. (2015). Comparison of the gut microbiota composition between obese and non-obese individuals in a Japanese population, as analyzed by terminal restriction fragment length polymorphism and next-generation sequencing. *BMC Gastroenterology*, *15*(1), 100.

Kassi, E., Pervanidou, P., Kaltsas, G., & Chrousos, G. (2011). Metabolic syndrome: Definitions and controversies. *BMC Medicine*, *9*(1), 48. doi: 10.1186/1741-7015-9-48.

Kent, S., Jebb, S. A., Gray, A., Green, J., Reeves, G., & Beral, V. On behalf of the Million Women Study Collaborators. (2019). Body mass index and use and costs of primary care services among women aged 55–79 years in England: A cohort and linked data study. *International Journal of Obesity*, *43*(9), 1839–1848. doi: 10.1038/s41366-018-0288-6.

Khalesi, S., Bellissimo, N., Vandelanotte, C., Williams, S., Stanley, D., & Irwin, C. (2019). A review of probiotic supplementation in healthy adults: Helpful or hype? *European Journal of Clinical Nutrition*, *73*(1), 24–37. doi: 10.1038/s41430-018-0135-9.

Kim, G. B., & Lee, B. H. (2008). Genetic analysis of a bile salt hydrolase in *Bifidobacterium animalis* subsp. *lactis* KL612. *Journal of Applied Microbiology*, *105*, 778–789.

Kirchner, H., Osler, M. E., Krook, A., & Zierath, J. R. (2013). Epigenetic flexibility in metabolic regulation: Disease cause and prevention? *Trends in Cell Biology*, *23*(5), 203–209. https://doi.org/10.1016/j.tcb.2012.11.008.

Kolehmainen, M., Mykkänen, O., Kirjavainen, P. V., Leppänen, T., Moilanen, E., Adriaens, M., et al. (2012). Bilberries reduce low-grade inflammation in individuals with features of metabolic syndrome. *Molecular Nutrition & Food Research*, *56*(10), 1501–1510.

Koliada, A., Syzenko, G., Moseiko, V., Budovska, L., Puchkov, K., Perederiy, V., et al. (2017). Association between body mass index and Firmicutes/Bacteroidetes ratio in an adult Ukrainian population. *BMC Microbiology*, *17*(1), 1–6.

Kong, C., Gao, R., Yan, X., Huang, L., & Qin, H. (2019). Probiotics improve gut microbiota dysbiosis in obese mice fed a high-fat or high-sucrose diet. *Nutrition*, *60*, 175–184. https://doi.org/10.1016/j.nut.2018.10.002.

Koutnikova, H., Genser, B., Monteiro-Sepulveda, M., Faurie, J.-M., Rizkalla, S., Schrezenmeir, J., et al. (2019). Impact of bacterial probiotics on obesity, diabetes and non-alcoholic fatty liver disease related variables: A systematic review and meta-analysis of randomised controlled trials. *BMJ Open*, *9*(3), e017995. doi: 10.1136/bmjopen-2017-017995.

Lau, E., Neves, J. S., Ferreira-Magalhães, M., Carvalho, D., & Freitas, P. (2019). Probiotic ingestion, obesity, and metabolic-related disorders: Results from NHANES, 1999–2014. *Nutrients*, *11*(7), 1482. doi: 10.3390/nu11071482.

Le Barz, M., Daniel, N., Varin, T. V., Naimi, S., Demers-Mathieu, V., Pilon, G., et al. (2019). In vivo screening of multiple bacterial strains identifies *Lactobacillus rhamnosus* Lb102 and *Bifidobacterium animalis* ssp. lactis Bf141 as probiotics that improve metabolic disorders in a mouse model of obesity. *The FASEB Journal*, *33*(4), 4921–4935.

Legrand, R., Lucas, N., Dominique, M., Azhar, S., Deroissart, C., Le Solliec, M. -A., et al. (2020). Commensal *Hafnia alvei* strain reduces food intake and fat mass in obese mice—A new potential probiotic for appetite and body weight management. *International Journal of Obesity*, *44*(5), 1041–1051. doi: 10.1038/s41366-019-0515-9.

Levy, M., Kolodziejczyk, A. A., Thaiss, C. A., & Elinav, E. (2017). Dysbiosis and the immune system. *Nature Reviews Immunology*, *17*(4), 219–232. doi: 10.1038/nri.2017.7.

Ley, R. E., Turnbaugh, P. J., Klein, S., & Gordon, J. I. (2006). Human gut microbes associated with obesity. *Nature*, *444*(7122), 1022–1023.

Li, Y.-F., Chang, Y.-Y., Huang, H.-C., Wu, Y.-C., Yang, M.-D., & Chao, P.-M. (2015). Tomato juice supplementation in young women reduces inflammatory adipokine levels independently of body fat reduction. *Nutrition*, *31*(5), 691–696.

Liong, M.-T., Lee, B., Choi, S. B., Lew, L. C., Lau, A., & Daliri, E. B.-M. (2015). Cholesterol-lowering effects of probiotics and prebiotics: A review of in vivo and in vitro findings. In Venema Koen, & do Carmo Ana Paula (Eds.), *Probiotics and Prebiotics: Current Research and Future Trends* (pp. 429–446). Caister Academic Press.

Liu, R., Hong, J., Xu, X., Feng, Q., Zhang, D., Gu, Y., et al. (2017). Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. *Nature Medicine*, *23*(7), 859–868. doi: 10.1038/nm.4358.

Lucas, N., Legrand, R., Deroissart, C., Dominique, M., Azhar, S., Le Solliec, M. A., et al. (2019). Hafnia alvei HA4597 strain reduces food intake and body weight gain and improves body composition, glucose, and lipid metabolism in a mouse model of hyperphagic obesity. *Microorganisms*, *8*(1), 35. doi: 10.3390/microorganisms8010035.

Majewska, K., Kręgielska-Narożna, M., Jakubowski, H., Szulinska, M., & Bogdanski, P. (2020). The multispecies probiotic effectively reduces homocysteine concentration in obese women: A randomized double-blind placebo-controlled study. *Journal of Clinical Medicine*, *9*, 998. doi: 10.3390/jcm9040998.

McFarland, L. V. (2014). Use of probiotics to correct dysbiosis of normal microbiota following disease or disruptive events: A systematic review. *BMJ Open*, *4*(8), e005047. doi: 10.1136/bmjopen-2014-005047.

Michael, D. R., Jack, A. A., Masetti, G., Davies, T. S., Loxley, K. E., Kerry-Smith, J., et al. (2020). A randomised controlled study shows supplementation of overweight and obese adults with lactobacilli and bifidobacteria reduces bodyweight and improves well-being. *Scientific Reports*, *10*(1), 4183. doi: 10.1038/s41598-020-60991-7.

Mitjavila, M. T., Fandos, M., Salas-Salvadó, J., Covas, M. I., Borrego, S., Estruch, R., et al. (2013). The Mediterranean diet improves the systemic lipid and DNA oxidative damage in metabolic syndrome individuals. A randomized, controlled, trial. *Clinical Nutrition*, *32*(2), 172–178. doi: 10.1016/j.clnu.2012.08.002.

Mullish, B. H., Michael, D. R., McDonald, J. A., Masetti, G., Plummer, S. F., & Marchesi, J. R. (2021). Identifying the factors influencing outcome in probiotic studies in overweight and obese patients: Host or microbiome? *Gut*, *70*(1), 225–226. doi: 10.1136/gutjnl-2020-321110.

Palaniappan, L. P., Wong, E. C., Shin, J. J., Fortmann, S. P., & Lauderdale, D. S. (2011). Asian Americans have greater prevalence of metabolic syndrome despite lower body mass index. *International Journal of Obesity (London)*, *35*(3), 393–400. doi: 10.1038/ijo.2010.152.

Pedret, A., Valls, R. M., Calderón-Pérez, L., Llauradó, E., Companys, J., Pla-Pagà, L., et al. (2019). Effects of daily consumption of the probiotic *Bifidobacterium animalis* subsp. *lactis* CECT 8145 on anthropometric adiposity biomarkers in abdominally obese subjects: A randomized controlled trial. *International Journal of Obesity*, *43*(9), 1863–1868. doi: 10.1038/s41366-018-0220-0.

Pitsavos, C., Panagiotakos, D. B., Tzima, N., Chrysohoou, C., Economou, M., Zampelas, A., et al. (2005). Adherence to the Mediterranean diet is associated with total antioxidant capacity in healthy adults: The ATTICA study. *The American Journal of Clinical Nutrition*, *82*(3), 694–699.

Principi, N., Cozzali, R., Farinelli, E., Brusaferro, A., & Esposito, S. (2018). Gut dysbiosis and irritable bowel syndrome: The potential role of probiotics. *Journal of Infection*, *76*(2), 111–120. https://doi.org/10.1016/j.jinf.2017.12.013.

Radd-Vagenas, S., Kouris-Blazos, A., Singh, M. F., & Flood, V. M. (2017). Evolution of Mediterranean diets and cuisine: Concepts and definitions. *Asia Pacific Journal of Clinical Nutrition*, *26*(5), 749–763.

Rodriguez, J., Hiel, S., Neyrinck, A. M., Le Roy, T., Pötgens, S. A., Leyrolle, Q., et al. (2020). Discovery of the gut microbial signature driving the efficacy of prebiotic intervention in obese patients. *Gut*, *69*(11), 1975–1987. doi: 10.1136/gutjnl-2019-319726.

Sagheddu, V., Guidesi, E., Galletti, S., & Elli, M. (2019). Selection and characterization criteria of probiotics intended for human use from the past to the future. *Food Science and Nutrition Studies*, *3*, 73. doi: 10.22158/fsns.v3n2p73.

Saini, R., Saini, S., & Sharma, S. (2010). Potential of probiotics in controlling cardiovascular diseases. *Journal of Cardiovascular Disease Research*, *1*(4), 213–214. doi: 10.4103/0975-3583.74267.

Saklayen, M. G. (2018). The global epidemic of the metabolic syndrome. *Current Hypertension Report*, *20*(2), 12. doi: 10.1007/s11906-018-0812-z.

Salas-Salvadó, Jordi, Bulló, Monica, Babio, Nancy, Martínez-González, Miguel Ángel, Ibarrola-Jurado, Núria, Basora, Josep, et al. (2011). Reduction in the incidence of type 2 diabetes with the Mediterranean diet: results of the PREDIMED-Reus nutrition intervention randomized trial. *Diabetes Care*, *34*(1), 14–19. doi: 10.2337/dc10-1288.

Salazar, N., Neyrinck, A. M., Bindels, L. B., Druart, C., Ruas-Madiedo, P., Cani, P. D., et al. (2019). Functional effects of EPS-producing bifidobacterium administration on energy metabolic alterations of diet-induced obese mice. *Frontiers in Microbiology*, *10*, 1809.

Sergeev, I. N., Aljutaily, T., Walton, G., & Huarte, E. (2020). Effects of synbiotic supplement on human gut microbiota, body composition and weight loss in obesity. *Nutrients*, *12*(1), 222. doi: 10.3390/nu12010222.

Shoushou, I. M., Melebari, A. N., Alalawi, H. A., Alghaith, T. A., Alaithan, M. S., Albriman, M. H. A., et al. (2020). Evaluation of metabolic syndrome in primary health care. *International Journal of Pharmaceutical Research and Allied Sciences*, *9*(1), 52–55.

Sun, L., Ma, L., Ma, Y., Zhang, F., Zhao, C., & Nie, Y. (2018). Insights into the role of gut microbiota in obesity: Pathogenesis, mechanisms, and therapeutic perspectives. *Protein & Cell*, *9*(5), 397–403. doi: 10.1007/s13238-018-0546-3.

Suzumura, E. A., Bersch-Ferreira, Â. C., Torreglosa, C. R., da Silva, J. T., Coqueiro, A. Y., Kuntz, M. G., et al. (2019). Effects of oral supplementation with probiotics or synbiotics in overweight and obese adults: A systematic review and meta-analyses of randomized trials. *Nutrition Reviews*, *77*(6), 430–450.

Tenorio-Jiménez, C., Martínez-Ramírez, M. J., Gil, Á., & Gómez-Llorente, C. (2020). Effects of probiotics on metabolic syndrome: A systematic review of randomized clinical trials. *Nutrients*, *12*(1), 124. doi: 10.3390/nu12010124.

Tierney, A. C., McMonagle, J., Shaw, D., Gulseth, H., Helal, O., Saris, W., et al. (2011). Effects of dietary fat modification on insulin sensitivity and on other risk factors of the metabolic syndrome—LIPGENE: A European randomized dietary intervention study. *International Journal of Obesity*, *35*(6), 800–809.

Torres, S., Fabersani, E., Marquez, A., & Gauffin-Cano, P. (2019). Adipose tissue inflammation and metabolic syndrome. The proactive role of probiotics. *European Journal of Nutrition*, *58*(1), 27–43.

Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Duncan, A., Ley, R. E., et al. (2009). A core gut microbiome in obese and lean twins. *Nature*, *457*(7228), 480–484.

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, *444*(7122), 1027.

Vähämiko, S., Laiho, A., Lund, R., Isolauri, E., Salminen, S., & Laitinen, K. (2019). The impact of probiotic supplementation during pregnancy on DNA methylation of obesity-related genes in mothers and their children. *European Journal of Nutrition*, *58*(1), 367–377. doi: 10.1007/s00394-017-1601-1.

Vessby, B., Uusitupa, M., Hermansen, K., Riccardi, G., Rivellese, A. A., Tapsell, L. C., et al. (2001). Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. *Diabetologia*, *44*(3), 312–319.

Von Bibra, H., Wulf, G., Sutton, M. S. J., Pfützner, A., Schuster, T., & Heilmeyer, P. (2014). Low-carbohydrate/high-protein diet improves diastolic cardiac function and the metabolic syndrome in overweight-obese patients with type 2 diabetes. *IJC Metabolic & Endocrine*, *2*, 11–18.

Xu, H., Li, X., Adams, H., Kubena, K., & Guo, S. (2018). Etiology of metabolic syndrome and dietary intervention. *International Journal of Molecular Sciences*, *20*(1), 128. doi: 10.3390/ijms20010128.

Yakoob, R. B. V. P. (2019). *Bifidobacterium* sp as probiotic agent—Roles and applications. *Journal of Pure and Applied Microbiology*, *13*, 1407–1417. doi: 10.22207/JPAM.13.3.11.

Yan, S., Tian, Z., Li, M., Li, B., & Cui, W. (2019). Effects of probiotic supplementation on the regulation of blood lipid levels in overweight or obese subjects: A meta-analysis. *Food & Function*, *10*(3), 1747–1759. doi: 10.1039/c8fo02163e.

Zafar, U., Khaliq, S., Ahmad, H. U., Manzoor, S., & Lone, K. P. (2018). Metabolic syndrome: An update on diagnostic criteria, pathogenesis, and genetic links. *Hormones (Athens)*, *17*(3), 299–313. doi: 10.1007/s42000-018-0051-3.

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Chapter 15

Bacillus Species—Elucidating the Dilemma on Their Probiotic and Pathogenic Traits

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1 Introduction

The human gut shelters diverse microbes that benefit the well-being of human health, as they are involved in nutritional, immunologic, and physiological functions of the host. Thus an imbalance of gut microbiota can cause acute and chronic diseases such as diarrhea, irritable bowel syndrome, inflammatory bowel disease, obesity, cancer, and autism. Therefore the rectification of gut health in a natural way formulates the rationale for probiotics therapy (Nicoleta, 2019; Markowiak & Śliżewska, 2017). Probiotics are nothing but live microorganisms when administered at a suitable volume confers health benefits to the host. Studies on probiotics were surpassing after the Russian noble laureate, Elie Metchnikoff who popularized the health benefits of beneficial microbes (Elshaghabee, Rokana, Gulhane, Sharma, & Panwar, 2017). Probiotic formulations have been developed and utilized extensively to improve human, poultry, aquaculture, and cattle health. Globally recognized strains of *Lactobacillus* (LAB), *Bifidobacterium*, *Saccharomyces*, *Streptococcus*, and *Enterococci* have been extensively explored for probiotic characteristics in several clinical diseases like diarrhea, inflammatory bowel disease, obesity, type 2 diabetes, cardiovascular disease, and cancer (Elshaghabee et al., 2017; Nicoleta, 2019). Thus it is understood that any beneficial strains that used to maintain the balance of gut microbiota would protect human health from diseases. Though many probiotic products of *Lactobacillus* and *Bifidobacterium* are clinically and commercially available, the scientific community does not stop working on new probiotics and still more species have been tested for their probiotics attributes to afford superior health benefits (Cutting, 2011).

One such sporeformer bacterial strain is *Bacillus* that has been consumed from fermented foods right from ancient periods. *Bacillus* denotes a Gram-positive, rod-shaped, spore-forming, aerobic, or facultative anaerobic bacterium and consists of greater probiotic attributes over nonspore-forming probiotics in terms of their stability at a higher acidic and wide range of temperature conditions. The scientific interest in *Bacillus* sp. as probiotics has only occurred in the last 15 years after the three principal reviews of (Mazza, 1994) and commercially known *Bacillus* probiotics include *B. subtilis*, *B. polyfermenticus*, *B. clausii*, *B. cereus*, *B. coagulans*, *B. pumilus*, and *B. licheniformis* (Sanders, Morelli, & Tompkins, 2003; Hong, Duc, & Cutting, 2005). Other than probiotic attributes, *Bacillus* sp. possesses greater advantages such as pathogen exclusion, antioxidant, antimicrobial, and immunomodulatory activities. Further, they have been used in the food factories and nutraceuticals for the production of extracellular enzymes such as food-grade amylase, glucoamylase, protease, pectinase, and cellulose and vitamin supplements for human consumption, respectively (Cutting, 2011). Despite all the advantages, few members of *Bacillus* sp., particularly *B. cereus*, *B. weihenstephanensis*, *B. anthracis*, and *B. thuringinesis*, are known as human pathogens that have the potential to produce toxins and transferable antibiotic resistance. These pathogenic issues and the free-living nature of *Bacillus* sp. in the gut augments the concern about the safety issues and leads to poor attention from the public regarding the commercial use of *Bacillus* probiotics (Elshaghabee et al., 2017).

This chapter discusses the probiotic and pathogenic attributes of *Bacillus* sp., advantages of sporeformers in the gut and food chain, health benefits of *Bacillus* probiotics, synbiotics of *Bacillus* sp., probable mechanism of action, commercial *Bacillus* sp. probiotic foods, and safety concerns.

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00015-0
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2 Advantages of sporeformers in the gut and food chain

The genus *Bacillus* is usually known as a group of soil residents. Yet, *Bacillus* sp. can be isolated from several sources, including air, water, human, animal intestines, and even food (Kotb, 2015; Tidjiani Alou et al., 2015). Looking to the probiotic prospect, it is proclaimed that the candidate should be isolated from the intestine of the target population, which helps them thrive well within the intestine. Elementary survivability attributes of native flora, however, are not essential for sporeformers because *Bacillus* spores can live in extreme stomach acidity and tolerate bile salts and other aggressive conditions of gastrointestinal tracts (GITs) (Sanders et al., 2003). Besides, *Bacilli* are more robust in the processing and storage of food and pharmaceutical preparations, making them an appropriate ingredient for the promotion of health formulations (Adewumi, Oguntoyinbo, Romi, Singh, & Jeyaram, 2014; Sanders et al., 2003).

The presence of *Bacillus* sp. in the gut must be associated with soil-related bacteria. A unified theory says that *Bacillus* sp. present in an endosymbiotic relationship with their host can temporarily survive and proliferate within the GIT. In some cases, endosymbiont like *B. anthracis*, *B. thuringiensis*, and *B. cereus* has evolved further as a pathogen, exploiting the gut as its primary portal of entry to the host for the synthesis of enterotoxins (Hong et al., 2005).

In vitro studies results suggest that the vegetative cells and spores of *B. cereus* may well defend and adhere to GIT stress and the intestinal epithelium. However, commensal intestinal microbiota has inhibitory activity against them (Elshaghabee et al., 2017). A study conducted by (Tam, 2006) showed *Bacillus* sp. spores could be recovered readily within the range of 10^3–10^8 cfu/g of human feces. The phylogenetic analysis of isolates from the 16S rRNA gene demonstrated the presence of 10 different *Bacillus* species in 30 volunteer fecal samples. The diversity of *Bacillus* sp. was further investigated by (Hoyles, 2012). Most of the recovered isolates belonged to the Bacillaceae family and related sporeformer bacteria in human feces. (Casula & Cutting, 2002) and Ghelardi et al. (2015) proved that oral administration of spores germinated in significant numbers in the jejunum and ileum, suggesting their colonization into the small intestine. Nyangale (Nyangale, 2014) observed that after 28-day treatment of *B. coagulans* in elderly subjects, baseline populations of *Faecalibacterium prausnitzii*, *Clostridium lituseburense*, and *Bacillus* sp. were significantly higher, relative to the placebo group. Likewise, a study by Adami & Cavazzoni (1999) in the piglet model has also shown that the feeding of *B. coagulans* CNCM I-1061 increased aerobic and anaerobic sporeformers, decreased *lactococci*, *enterococci*, *anaerobic cocci*, and fecal coliforms in the treatment group.

Intake of vegetative cells and spores of *Bacillus* sp. through fermented foods and raw vegetables are common in humans. A wide variety of *Bacillus* species were found to be associated with soy, locust, corn, rice, and many more substrates being fermented naturally. For example, Natto (Japan), Gari (Africa) TapaiUbi (Malaysia), Douchi (China), Rabadi (India, Pakistan), Soibum (India), and Ugba (Nigeria) are among the popular functional foods naturally harboring the blend of *Bacillus* sp. and *Lactobacillus* (Cutting, 2011; Lee, Kim, & Paik, 2019). These fermented products exhibit specific qualities, possibly due to extracellular carbohydrate and protein degrading enzymes produced by *Bacillus* sp. A diverse array of LAB and *Bacillus* sp. isolated from these indigenous foods has researched industries for commercial purposes. The strains of *B. subtilis*, *B. subtilis var. natto*, *B. clausii*, *B. licheniformis*, and *B. coagulans* are used internationally to boost the quality and demand of functional foods (Cutting, 2011; Elshaghabee et al., 2017; Lee, Kim, & Paik, 2019).

3 Probiotic attributes of *Bacillus* species

Generally, bacterial spores are produced in extreme environmental conditions to enable the long-term survival of vegetative bacteria. The structural features of the bacterial endospores consist of a core with inactive chromosomes, cortex that is rich in peptidoglycan, and spore coat rich in pretentious material. These structural features allow the spores to withstand harsh conditions such as UV irradiation, extreme heat conditions, a wide range of solvents, a verity of enzymes such as lysozyme (Driks, 1999; Henriques & Moran, 2007; Moir, 2006; Nicholson, Munakata, Horneck, Melosh, & Setlow, 2000). *Bacillus* sp., such as *B. laevolacticus* DSM 6475 and *B. racemicus* IAM 12395, were found to endure pH 2.5 for as long as 6 hours (Hyronimus, Le Marrec, Sassi, & Deschamps, 2000). *B. licheniformis* Me1, *Bacillus flexus* Hk1, and *B. subtilis* Bn1 isolated from milk, cheese, and fermented beans have shown better acid, bile tolerance, bile salt hydrolase activity, adhesion to hydrocarbons and autoaggregation properties, high antioxidant activity, and a broad spectrum of activity against food-borne pathogens (Lee et al., 2015). Likewise, *Bacillus racemilacticus* and *B. coagulans* strains were accounted to be lenient to bile fixations over 0.3% (w/v) (Ghelardi et al., 2015). These examinations showed high corrosive resistance of *Bacillus* sp., yet, the equivalent was flawed for bile resilience for scarcely any strains. Although the use of sporeformers as probiotics has not been all around contemplated, the resistance of spores

to natural pressure is an alluring characteristic for business applications, particularly in animal health and agribusiness industries. In contrast to other probiotic microscopic organisms, sporeformers are available in business items as spores and are not devoured as vegetative cells (Hong et al., 2005). Like nonspore shaping *Lactobacillus* sp., the endurance pace of spore-forming *Bacilli* is strain specific. Furthermore, the food network additionally assumes a significant job in the endurance of probiotics during recreated gastric juice conditions.

The components behind the beneficial properties of *Bacillus* sp. probiotics are antimicrobial, antioxidative, and immune-modulatory activities through the synthesis of antimicrobial peptides, small extracellular molecules, and their ability to interact with the host through adhesion and attachment features (Urdaci & Pinchuk, 2004). Also, *B. subtilis* strains have been reported to secrete amicoumacin A antibiotic against pathogens like *Helicobacter pylori*, *Enterococcus faecium*, and *Shigella flexneri* (Pinchuk et al., 2001). *B. clausii* secretes serine protease(s) against *Clostridium difficile* (Ripert et al., 2016). Rejection of pathogen by the restraint of bacterial biofilm is another potential property proposed for *Bacillus* strains. Bacteriocin producing strains of *B. subtilis* displayed a high level of antimicrobial activity against foot ulcers with highest recorded against *Klebsiella* sp. (Joseph, Dhas, Hena, & Raj, 2013). Such bacteriocin producing strains of *Bacillus* sp. has the potential to be introduced as food biopreservative and as an antimicrobial in human and animal infections.

Previous studies on health benefits of *Bacillus* sp. have been consolidated and presented in Table 15.1 (Elshaghabee et al., 2017; Hong et al., 2005; Lee, Kim, & Paik, 2019). Also, the antibiotics and secretary enzymes produced by *Bacillus* sp. are listed in Table 15.2 (Elshaghabee et al., 2017; Mannanov & Sattarova, 2001; Urdaci & Pinchuk, 2004).

TABLE 15.1 Primary health benefits of *Bacillus* sp. probiotics.

Name of *Bacillus* sp.	Benefits
B. clausii	1. Immunomodulatory and antimicrobial properties 2. Antiinflammatory effect against the side effects of antibiotic-based *Helicobacter pylori* therapy (Nista, 2004), and therapeutic effect against urinary tract infection 3. Demonstrate increased interferon production, mitogenic T cell proliferation, and mitogen-induced lymphokine production 4. Modulate immune response in allergic children with recurrent respiratory infections
B. coagulans	1. Produce coagulin, a bacteriocin, which has antimicrobial effect against a broad spectrum of enteric microbes 2. Generally recognized as safe (GRAS) by the Food and Drug Administration (FDA) 3. Reduces the blood lipid concentration 4. Adjunct therapy for rheumatoid arthritis
B. licheniformis	Antiobesity and antidiabetic properties and also a reduced accumulation of β-amyloid in the brain hippocampus
B. polyfermenticus	1. Cholesterol-reducing and antioxidant activities 2. Produces polyfermenticin SCD, a bacteriocin, which has an antimicrobial effect against *Staphylococcus aureus* KCCM 32359, *Clostridium perfringeins* ATCC 3624, and *H. pylori* 3. The anticarcinogenic effect of *B. polyfermenticus* SCD was investigated in human colon cancer cells, including HT-29, DLD1, and Caco-2 cells
B. subtilis	1. Secrete quorum-sensing pentapeptide, competence, and sporulation factors 2. Antimicrobial and anticancer activity 3. GRAS by the FDA 4. Inhibits the adhesion of *Salmonella enteritidis*, *Listeria monocytogenes*, and *Escherichia coli* to the HT-29 cells 5. Secretion of serine protease known as nattokinase reduces blood clotting by fibrinolysis 6. Produces antibiotics, including amicoumacin A and nonamicoumacin against *H. pylori* 7. Antiviral activity against influenza virus, herpes virus, and equine encephalomyelitis virus 8. Reported to produce γ-aminobutyric acid (GABA) that helps anxiety inhibition, sleep promotion, reducing blood pressure, and enhancement of immune response
B. pumilus	Decrease coliform counts in feces
B. subtilis var. natto	Secretes surfactin antibiotic to inhibit the growth of *Candida albicans* in the intestinal tract
B. firmus	Stimulate the proliferation of human peripheral blood lymphocytes in vitro

TABLE 15.2 Primary antibiotics and extracellular enzymes produced by *Bacillus* probiotics.

Bacillus sp.	Extracellular molecules	Type	Properties
B. brevis and *B. subtilis*	Gramicidin C, B, tyrocidine	Nonribosomal polypeptide	Antibiotic
B. licheniformis	Bacitracin	Peptide	Antibiotic
	Lichenin		Lantibiotics
B. subtilis	Mycobacillin	Nonribosomal cyclic	Antifungal
	Surfactin	Nonribosomal lipopeptides	Surfactant
	Bacilysin	Dipeptide	Antibiotic
	Subtilin	Ribosomal synthesis	Antibiotic and lantibiotics
	Nisin and Pep5	Lantibiotics	Membrane pore formation
	Amicoumacin A and nonamicoumacin	Isocoumarin and lipopeptide	Antibiotic
	Bacilysocin	Phospholipid	Antibiotic
	Sublancin	Peptide	Lantibiotic
	Ericins A, S	Peptide	Lantibiotic
	Mersacidin	Peptide	Lantibiotic
	Subtilisin	Cyclic peptide	Antibiotic
	Iturins (A, C, D, E), mycosubtilin, bacillomycins (D, F, L), bacillopeptin, fengycin	Lipoheptapeptide	Antifungal
	Rhizocticin	Phosphono-oligopeptide	Antifungal
B. laterosporus	Loloatin A and tupuseleiamid	Cyclic decapeptide and acyldepsipeptide	–
B. subtilis and *B. cereus*	Fengycin, plipastatins (A1, A2)	Lipodecapeptide	Antifungal
B. coagulans, *B. subtilis*, *B. cereus*, *B. licheniformis*, *B. amyloliquifaciens*, *B. megaterium*, *B. caldolyticus*, *B. polymyxa*, *B. pumilus*, *B. circulans*, *B. firmus*, *B. brevis*, *B. macerans*, and *B. stearothermophilus*	α-Amylase, β-amylase, arabinase, cellulase, chitinase, chitosanase, dextranase, galactanase, β-1,3-glucanase, β-1,6-glucanase, isoamylase, lichenase, levansucrase, maltase, mannanase, pectolyase, phosphomannose, pullulanase, xylanase, glucose isomerase	Ribosomal synthesis	Carbohydrate degrading enzymes
B. subtilis, *B. cereus*, *B. licheniformis*, *B. amyloliquifaciens*, *B. megaterium*, *B. polymyxa*, *B. thermoproteolyticus*, *B. thuringiensis*, *B. pumilus*	Aminopeptidase, esterase, metal proteases, serine protease	Ribosomal synthesis	Protease
B. licheniformis, *B. cereus*, *B. anthracis*, *B. thuringiensis*, *B. thiaminolyticus*	Phospholipase C, thiaminase	Ribosomal synthesis	Lipase
B. amyloliquifaciens, *B. subtilis*, *B. cereus*, *B. megaterium*, *B. pumilus*	Deoxyribonuclease, ribonucleases, 3-nucleotidases, 5-nucleotidases	Ribosomal synthesis	Nuclease
B. amyloliquifaciens, *B. subtilis*, *B. cereus*	Alkaline phosphatase	Ribosomal synthesis	Phosphatase

4 Synbiotics of *Bacillus* sp.

Synbiotics—as the name suggests, these are the combination of probiotics and prebiotics to provide a synergistic effect to humans for stabilizing the GIT or gut flora and this was first proposed by Gibson and Roberfroid (Gibson, Probert, Van Loo, Rastall, & Roberfroid, 2004). The gut flora comprises thousands of microbial strains distributed mainly in the colon, stomach, and ileum to encounter the invading pathogens, thus producing antimicrobial substances, including bacteriocin. The gut flora also engages in other physiological functions, including the production of vitamin, synthesis of amino acids, biotransformation of bile, preventing colonization of pathogens, and actively involving in promoting immune responses (Vyas & Ranganathan, 2012). Stabilization of gut is one important aspect in humans as they are much affected by several factors, including unhealthy diet, age, stress, repeated exposure to antibiotics, and so on, thus ruining the normal physiological functions. Some of the ill effects caused by gut imbalance are antibiotic-associated diarrhea, irritable bowel syndrome, hypercholesterolemia, colon cancer, and many more. Probiotics are the group of good microorganisms (mainly bacteria) that are capable of conferring beneficial effect to the consumers when provided in sufficient amounts. Probiotics can be supplied individually or in a mixture to exhibit such health benefits. The major issue with probiotics is that they suffer survival difficulties in passing through the human intestinal tract due to several factors like oxygen, pH, organic acids, and H_2O_2. Prebiotics are mainly fibers or nondigestible carbohydrates which when combined with probiotics can act as food for the bacteria so that, they can grow and multiply in the intestine. The prebiotic compounds should selectively promote the growth of probiotics and not the other microorganisms. Hence, the selection of the right probiotics and prebiotics must be prioritized on the basis of the host's health, as the synbiotic formulations act differently on the basis of the patient group. This chapter will detail the basics of synbiotics and will be addressing the need for synbiotics and their mechanisms of action related to human health, with a special focus toward *Lactobacillus*-based synbiotic combinations (Krumbeck, Walter, & Hutkins, 2018; Markowiak & Śliżewska, 2017).

5 The rationale to use synbiotics

A synbiotic product affects the host favorably by selectively stimulating the growth of specific or a limited number of health-promoting bacteria thereby improving their survival and implantation in the gut. As mentioned earlier, the main aim of developing synbiotics is to overcome the survival difficulties of probiotics as probiotics are generally active in small and large intestines.

The rationale behind the use of synbiotics is to beneficially affect the host in the following ways (Gyawali et al., 2019; Pandey, Naik, & Vakil, 2015):

1. Improving the survival or viability of the selected single or multiple but limited probiotic bacteria during the passage through the intestinal tract.
2. Effectuate the implantation of the health-promoting probiotics in the colon to stimulate the effect of its growth to maintain the intestinal homeostasis.
3. Stimulating the gut immunity in host.

Role of probiotics: The probiotics improve the health status of the host in combination with prebiotics by

1. maintaining the intestinal microbiota by resisting colonization and suppressing pathogenic microorganisms in the body thereby improving the digestion process;
2. improving the metabolic reactions in the body mainly by decreasing the gut toxin level to fight diarrhea. This also includes the reduction of serum cholesterol level by deconjugation and secretion of bile salt, facilitating lactose digestion, and providing the required nutrients; and
3. providing enhanced immunomodulatory effects.

Role of prebiotics: The prebiotics are not only involved in growth promotion of the combined probiotics but also in the following functions:

1. Maintain the intestinal biostructure
2. Protect infection by concomitant inhibition of pathogens
3. Inhibition of carcinogenesis (mainly colorectal cancer)
4. Supporting the immune system by immunomodulatory effects
5. Nutrient absorption to combat metabolic syndrome and obesity

The list of synbiotics of *Bacillus* sp. related to human health has been summarized in Table 15.3 and synbiotics related to aquaculture have been detailed in? (Ringø & Song, 2016).

TABLE 15.3 Primary beneficial effects of *Bacillus* synbiotics.

Composition of synbiotics	Subject and time of administration	Outcome	References
Lactobacillus plantarum, *Bacillus Coagulans*, and inulin	24 male Wistar rats/21 days and 42 days	Reduce the level of cadmium in the tissues, preventing liver and kidney damage and recover antioxidant enzymes in acute cadmium poisoning in the rat	Jafarpour et al. (2017)
Lactol composed of *B. coagulans* and fructo-oligosaccharides	Iranian irritable bowel syndrome patients/4–8 weeks	Effective in relieving abdominal pain/discomfort and diarrhea, but not constipation in IBS patients	Rogha, Esfahani, and Zargarzadeh (2014)
Bacillus subtilis and dahlin	Weaner piglets/2 weeks	Enhance the growth performance and humoral immune functions	Xianjun and Fei (2012)
B. coagulans and inulin	Wistar rats/4 weeks	Efficient in beneficially modulating GI microbiota and decrease total cholesterol and LDL-cholesterol in serum Improve the biochemical and clinical parameters of induced rheumatoid arthritis in rat	Abhari, Shekarforoush, Sajedianfard, Hosseinzadeh, and Nazifi (2015)
B. coagulans and green banana resistant starch	Mice/1 week	Ameliorated the overall inflammatory status of the experimental inflammatory bowel disease model	Shinde et al. (2020)
B. coagulans lilac-01 and okara [soy pulp] powder	Healthy Japanese volunteers with a tendency for constipation/2 weeks	Improved the bowel movements and fecal properties	Minamida, Nishimura, Miwa, and Nishihira (2015)
B. subtilis and inulin	Lohmann White laying hens	Improved egg production and eggshell quality of laying hens	Abdelqader, Al-Fataftah, and Daş (2013)
B. subtilis HMNig-2 and levan *B. subtilis* MENO2 and levan	Mouse	Reduce the coliform count by 1.5 logs while increasing *Lactobacillus* bacteria count in the gut by almost 0.8 logs. Protect the liver from *Salmonella typhimurium* complications	Hamdy et al. (2018)
B. subtilis and acanthopanax polysaccharide biostime	Brown chicks/7 week	Good effect on the ileum organization structure and duodenum digestive enzymes of chicks	Zhao, Shao, and Gong (2015)
MegaSporebiotic, a proprietary probiotic mixture of *Bacillus indicus* (HU36), *B. subtilis* (HU58), *B. coagulans* SC-208, *Bacillus licheniformis*, and *Bacillus clausii* SC-109 spores, and MegaPrebiotic, a proprietary prebiotic blend of fructo-oligosaccharides (FOS) from green and gold kiwifruit (Livaux™ and ACTAZIN™), xylooligosaccharides (XOS) from corn cob (PreticX™) and galactooligosaccharides (GOS) from cow milk (Bimuno®)	Simulator of the human intestinal microbial ecosystem/4 weeks	A mixture of five spore-forming *Bacillus* strains and a prebiotic blend of FOS, GOS, and XOS, consistently affected microbial activity and composition in the human gastrointestinal tract in vitro, with profound effects being observed on colonic butyrate production	Duysburgh, Van den Abbeele, Krishnan, Bayne, and Marzorati (2019)
Saccharomyces boulardii, *B. coagulans*, and fructo-oligosaccharide	Nursing home residents undergoing antibiotic therapy/2 weeks	Safe approach to control antibiotic-associated diarrhea and *Clostridium difficile* infection	Spielholz (2011)
Probiotics (*B. subtilis*, *B. licheniformis*, and *Clostridium butyricum*) and prebiotics (yeast cell wall and xylooligosaccharide)	Broilers	An effective method for improving growth performance and carcass compositions, resulting in the production of meat with favorable quality and oxidative stability	Cheng et al. (2017)

6 Mechanism of action of *Bacillus* probiotics

Bacillus sp., as mentioned earlier, is a group of gut commensal microorganisms present in humans and animals and is presumed as probiotics for decades (Sanders et al., 2003). These species are capable of entering the host through ingestion, surviving, and proliferating in the gut of host by endosymbiotic relationship temporarily. Based on several clinical trials and researches, the roles of *Bacillus* sp. as probiotics have been postulated. The majorly examined *Bacillus* strains for their probiotic activity in humans include *B. subtilis*, *B. clausii*, *B. licheniformis*, *B. polyfermenticus*, and *B. coagulans* (Hong et al., 2005). Apart from survival, implantation, and exertion of intestinal homeostasis, these probiotics render vast physiological functions in the host.

The mechanisms of action of spore forming probiotics (SFP), especially the *Bacillus* probiotics, to exert probiotic action can be different in the gut environment when compared to the species' regular life cycle. The spore-based probiotic products are being evaluated widely for their probiotic property as the spores are highly stable and resistant, that is, the spore is capable of withstanding UV rays, extreme heat (even up to 85°C), exposure to hydrogen peroxide, lysozyme, and is capable of growing, proliferating, and resporulating in the small intestine amidst its hostile conditions (Nicholson et al., 2000). The effective mechanisms by which probiotics improve the health status of the host, as proposed by many authors are discussed next (Markowiak & Śliżewska, 2017).

7 Mechanism 1—antimicrobial activity

The *Bacillus* probiotics maintain the intestinal microbiota by synthesizing different antimicrobials and enzymes (mainly exoenzymes) (Fig. 15.1) thereby suppressing the pathogenic microorganisms in the host. They involve in competitive prohibition and improve the metabolic reactions in the body by nutrient absorption and help combat metabolic syndrome. Apart from this, they effectuate the health-promoting probiotics in the gut to stimulate the effect of their growth to maintain the intestinal homeostasis by the production of certain vitamins and peptides.

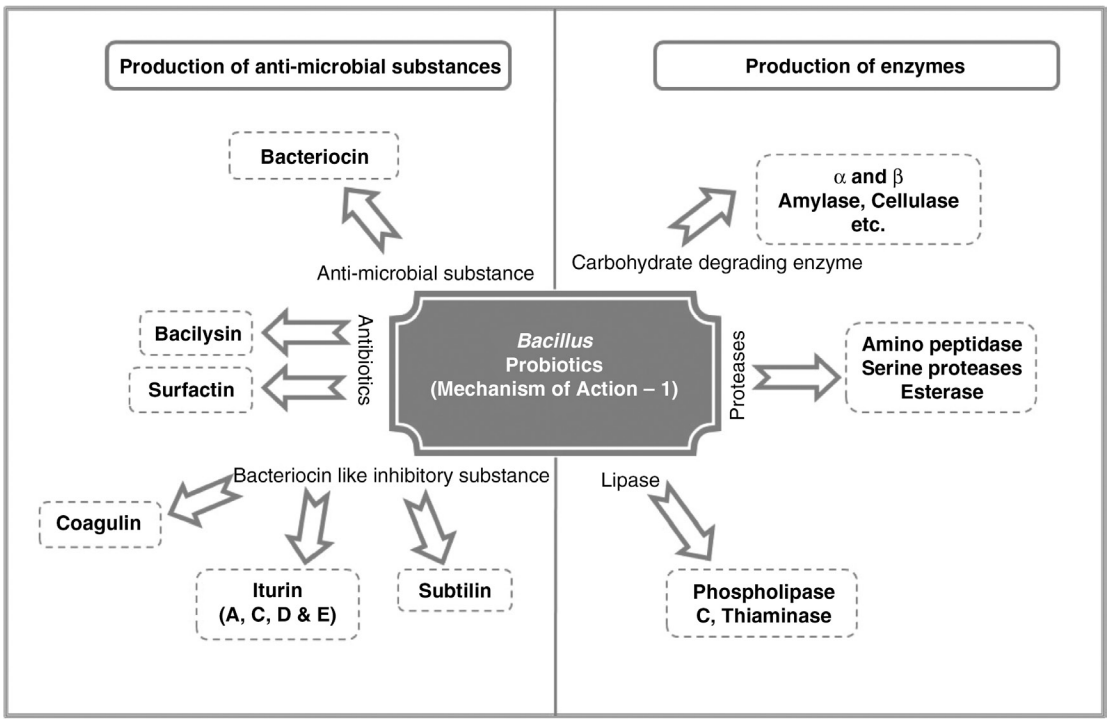


FIGURE 15.1 First mechanism of action of *Bacillus* probiotics.

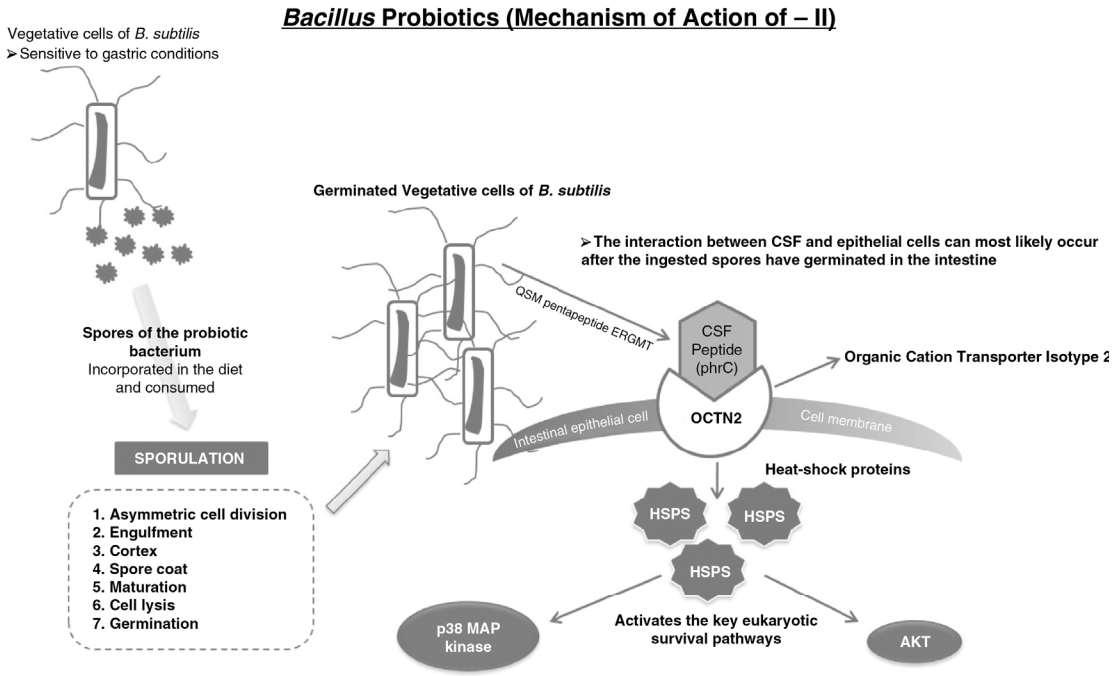


FIGURE 15.2 Second mechanism of action of *Bacillus* probiotics.

8 Mechanism 2—interaction with intestinal and immune cells

Though the interactions of *Bacillus* probiotics with the native or the intestinal microorganisms and with the host immune cells are not clearly understood, the interaction does happen to develop the gut-associated lymphoid tissues (GALT) and immune cells (mainly some spore-specific IgA and IgG responses). Based on some interesting research outcomes, it is expected to happen for the following two things:

1. To influence the functions of the gut microflora
2. To persuade the host's response required for the intestinal stability

Generally, bacteria generate more number of metabolites that can be recognized by host cells. Bioactive peptides like quorum sensing molecules are produced by bacteria (both Gram-positive and Gram-negative) to combat the harsh environment in the host (Fujiya et al., 2007). The existence of such metabolite-based cell communication has been examined in *B. subtilis* against human intestinal like (Caco-2) cells (Fig. 15.2). Some mutant strains of *B. subtilis* have shown mere or no development of GALT due to the inability to produce spores.

Though sporulation plays a major role in probiotic effect, not all sporulating *Bacillus* strains (e.g., *B. licheniformis*, *B. pumilus*) enhance the development of GALT and antibodies (mainly the preimmune antibodies) in the host (Rhee, Sethupathi, Driks, Lanning, & Knight, 2004). The vegetative form of *Bacillus* probiotics is recognized by major Toll-like receptors (TLR2 and 4) and stimulates B cell proliferation, while the spores are considered immunogenic and are capable of stimulating a cellular immune response (mainly IFN-γ) (Huang, 2008).

9 Commercially available *Bacillus* probiotics

Functional foods like probiotics are eminent tools in maintaining human and animal health by acting as alternatives in reducing the risks associated with several diseases thus preventing those diseases. Various studies have revealed the potential of probiotics in alleviating diseases related to human and animal intestine by maintaining the gut microflora. The principal and most acknowledged beneficial effects of probiotics range from diarrhea to cancer, including some serious issues like cardiovascular disease and autoimmune disease. In addition to the commonly explored strains (*Lactobacillus* and *Bifidobacterium)*, SFP, mostly of the genus *Bacillus* have shown potential probiotic functionalities. *Bacillus* sp. has considerably been used for the production of food-grade extracellular enzymes (like amylase, glucoamylase, and cellulase) and additional nutraceuticals (including vitamins like riboflavin, inositol, and some carotenoids) for the production of many health

TABLE 15.4 List of some commercially available *Bacillus* probiotics for human.

S. no.	Product	Components	Origin
1.	Anaban™	*Bacillus subtilis* PB6	Europe
2.	Bactisubtil®	*Bacillus cereus* strain IP 5832	Germany
3.	Bibactyl	*B. subtilis*	Vietnam
4.	Bidisubtilis	*B. subtilis*	Vietnam
5.	Bio-Kult	*B. subtilis* PXN21, *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* strains	United Kingdom
6.	Biosporin®	*B. subtilis* 2335/*B. subtilis* 3 and *Bacillus licheniformis* 2336	Ukraine
7.	Biosubtyl	*B. cereus* labeled as *B. subtilis*	Vietnam
8.	Biovicerin®	*B. cereus*	Brazil
9.	Biospan®	*Bacillus polyfermenticus* SCD	South Korea
10.	Domuvar	*Bacillus clausii* labeled as *B. subtilis* (no longer marketed)	Italy
11.	Enterogermina®	*B. clausii* labeled as *B. subtilis*	Italy
12.	Flora-Balance	*Bacillus laterosporus* (BOD)	United States
13.	Flora3	*Bacillus coagulans*, *Saccharomyces boulardii*, and fructo-oligosaccharides (FOS)	United States
14.	GanedenBC^{30}	*B. coagulans*	United States
15.	Just Thrive	*Bacillus indicus* HU36, *B. coagulans*, *B. clausii*, *B. subtilis* HU58	United States
16.	Lactipan Plus	*B. subtilis* labeled as *Lactobacillus sporogenes*	Italy
17.	Lactopure	*B. coagulans* labeled as *L. sporogenes*	India
18.	Lactospore	*B. coagulans* labeled as *L. sporogenes*	United States
19.	LifeinU™	*B. subtilis* CU1	Europe
20.	Medilac	*B. subtilis* with *Enterococcus faecium*	China
21.	MegaSporeBiotic	*B. indicus*, *B. subtilis*, *B. coagulans*, *B. licheniformis*, *B. clausii*	United Kingdom
22.	Nature's First Food	42 species listed as probiotics including *B. subtilis*, *Bacillus polymyxa*, *Bacillus pumilus*, and *B. laterosporus*	United States
23.	Neolactoflorene	A valid name is *B. coagulans* is mislabeled and is a strain of *B. subtilis*	Italy
24.	NutriCommit	*B. subtilis*, *B. coagulans*	United States
25.	Pastylbio	*B. subtilis*	Vietnam
26.	Primal Defense™	*B. subtilis* and *B. licheniformis*	Taiwan
27.	Subtyl	*B. cereus* species termed *B. cereus* var. *vietnami* labeled as *B. subtilis*	Vietnam
28.	Sunny Green	*B. coagulans*	United States
29.	THORNE®	*B. coagulans*	United States
30.	Vital Probiotics	*B. subtilis*, *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Bifidobacterium breve*	United States

supplements and/or prophylactics for human consumption. A list of some commercially available *Bacillus* probiotics in the global market for human use is given in Table 15.4 (Elshaghabee et al., 2017; Hong et al., 2005).

10 Pathogenic attributes of *Bacillus* sp.

Dangers of *Bacillus* probiotics incorporate enterotoxin secretion, antibiotic resistance, cytotoxicity against typical cells, and secretion of biogenic amine. Accordingly, probiotic strains must be examined to guarantee the well-being of their phenotypes and genotype qualities. Two sporeformers, *B. anthracis, and B. cereus*, are known as human pathogens. The previous requires no elaboration, while the utilization of *B. cereus* has all the earmarks of being a reason for worry dependent upon the situation.

The utilization of *B. cereus* probiotics has represented the danger of enterotoxin production and diarrhea-type or emetic-type disease. The accompanying qualities further accentuate the requirement for the safety assessment of these microscopic organisms: enterotoxin qualities, for example, hemolysin, nonhemolytic enterotoxin, and emetogenic toxin. A few *B. cereus* probiotics, such as Bactisubtil, Biosubtyl^{DL}, and Subtyl, have been reported for their enterotoxin production (Kotiranta, Lounatmaa, & Haapasalo, 2000; Lund & Granum, 1997). In addition, *B. cereus*, *B. licheniformis*, and *B. subtilis* have been reported in cases of foodborne diarrheal illness, toxin production, and vomiting (Hong et al., 2005). *Bacillus* strains have been listed as resistant/susceptible to all antibiotics except ampicillin (EFSA European Food Safety Authority, 2012). Four probiotic strains of *B. clausii* are marketed as an over-the-counter medical supplement and have been investigated for antibiotic resistance by antibiotic susceptibility assay, PCR amplification, and DNA sequencing. *B. licheniformis* cy2, *B. polyfermenticus* CJ9, *B. licheniformis* KCTC 1918, and *B. subtilis* KCTC 1028 are reported for biogenic amine production of putrescine, cadaverine, spermidine, phenylethylamine, and spermine (Chang & Chang, 2012).

Bacillus species' debate about probiotic versus pathogen tag is persisting in the fields of research and production. It is therefore critical that the phenotypic and genotypical characteristics of selective *Bacillus* sp. are understandable. And their identification with those of GRAS rank to build consensus on the same (Elshaghabee et al., 2017; Hong et al., 2005).

11 *Bacillus* probiotics—safety

Bacillus species apart from *B. anthracis* and *B. cereus* which are considered human pathogens are widely used in the production of fermented food products (like beans, antibiotics, enzymes). *Bacillus* probiotics are generally consumed as bio-therapeutics in the form of viable microbes and/or as fermented products. Hence, there is always a need for rigorous evaluation of these probiotics regarding their safety before marketing. The Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) guidelines for the evaluation of probiotics for consumption are given in Fig. 15.3.

The safety concern of *Bacillus* probiotics has widely been reviewed. Some misdiagnosis had been made on the adverse effects of *Bacillus* probiotics, back then. Though some *B. cereus* probiotics have shown enterotoxin production, various species of *Bacillus*, including *B. coagulans*, *Bacillus indicus* (Endres et al., 2009), *B. subtilis var. natto* (Hong et al., 2008), *B. subtilis* 2335, PY79, BS3 (Sorokulova et al., 2008), and *B. licheniformis* 2336, BL31 (Sorokulova et al., 2008), have been extensively studied using *in vitro* as well as in vivo (mainly acute and subchronic toxicity) studies and has been proposed to be safe apart from some opportunistic infections.

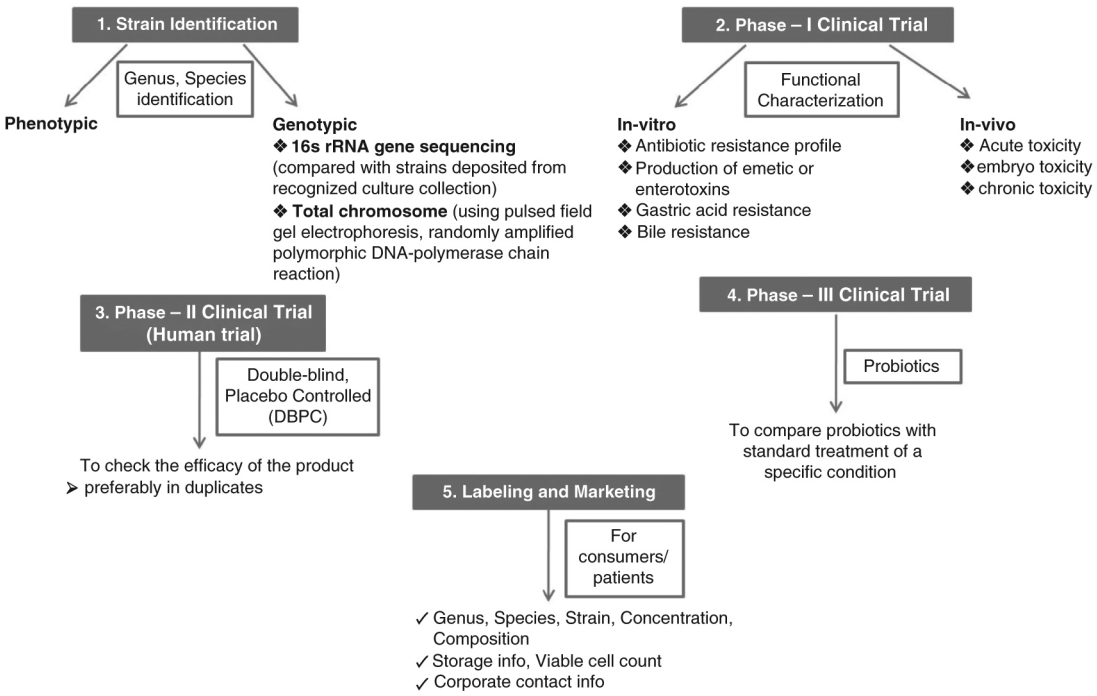


FIGURE 15.3 FAO/WHO guidelines for probiotics evaluation.

12 Conclusion

The use of *Bacillus* sp. as a probiotic supplementary product has been rapidly increasing in poultry and aquaculture industries. The spore-forming capacity of *Bacillus* sp. along with antimicrobial, antioxidant, and immunomodulatory activities becomes advantageous. Besides, these strains can be easily prepared as a stable and edible form in the industries for the benefit of human health. Also, additional studies on synbiotics of *Bacillus* sp. are needed to improve the beneficial effects with other probiotics and prebiotics. Further, focus on the phenotypical and genotypical characteristics of certain promising *Bacillus sp.* can uncover the dilemma on their safety concerns, helping them in becoming a benign species, safe for human and animal use shortly.

References

Abdelqader, A., Al-Fataftah, A. -R., & Daş, G. (2013). Effects of dietary *Bacillus subtilis* and inulin supplementation on performance, eggshell quality, intestinal morphology and microflora composition of laying hens in the late phase of production. *Animal Feed Science and Technology*, *179*, 103–111.

Abhari, K. H., Shekarforoush, S. S., Sajedianfard, J., Hosseinzadeh, S., & Nazifi, S. (2015). The effects of probiotic, prebiotic and synbiotic diets containing *Bacillus coagulans* and inulin on rat intestinal microbiota. *Iranian Journal of Veterinary Research*, *16*, 267.

Adami, A., & Cavazzoni, V. (1999). Occurrence of selected bacterial groups in the faeces of piglets fed with Bacillus coagulans as probiotic. *Journal of Basic Microbiology: An International Journal on Biochemistry, Physiology, Genetics, Morphology, and Ecology of Microorganisms*, *39*(1), 3–9.

Adewumi, G. A., Oguntoyinbo, F. A., Romi, W., Singh, T. A., & Jeyaram, K. (2014). Genome subtyping of autochthonous *Bacillus* species isolated from Iru, a fermented Parkiabiglobosa seed. *Food Biotechnology*, *28*, 250–268.

Casula, G., & Cutting, S. M. (2002). Bacillus probiotics: spore germination in the gastrointestinal tract. *Applied and Environmental Microbiology*, *68*(5), 2344–2352. doi: 10.1128/aem.68.5.2344-2352.2002.

Chang, M., & Chang, H. C. (2012). Development of a screening method for biogenic amine producing Bacillus spp. *International Journal of Food Microbiology*, *153*(3), 269–274. doi: 10.1016/j.ijfoodmicro.2011.11.008.

Cheng, Y., Chen, Y., Li, X., Yang, W., Wen, C., Kang, Y., et al. (2017). Effects of synbiotic supplementation on growth performance, carcass characteristics, meat quality and muscular antioxidant capacity and mineral contents in broilers. *Journal of the Science of Food and Agriculture*, *97*, 3699–3705.

Cutting, S. M. (2011). *Bacillus* probiotics. *Food Microbiology*, *28*, 214–220. https://doi.org/10.1016/j.fm.2010.03.007.

Driks, A. (1999). *Bacillus subtilis* spore coat. *Microbiology and Molecular Biology Reviews*, *63*, 1–20. https://doi.org/10.1128/MMBR.63.1.1-20.1999.

Duysburgh, C., Van den Abbeele, P., Krishnan, K., Bayne, T. F., & Marzorati, M. (2019). A synbiotic concept containing spore-forming *Bacillus* strains and a prebiotic fiber blend consistently enhanced metabolic activity by modulation of the gut microbiome in vitro. *International Journal of Pharmaceutics: X*, *1*, 100021.

Elshaghabee, F. M. F., Rokana, N., Gulhane, R. D., Sharma, C., & Panwar, H. (2017). *Bacillus* as potential probiotics: Status, concerns, and future perspectives. *Frontiers in Microbiology*, *8*, 1490. https://doi.org/10.3389/fmicb.2017.01490.

EFSA (European Food Safety Authority) (2012). Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. *EFSA Journal*, *10*(6), 2740. doi: 10.2903/j.efsa.2012.2740.

Endres, J. R., Clewell, A., Jade, K. A., Farber, T., Hauswirth, J., & Schauss, A. G. (2009). Safety assessment of a proprietary preparation of a novel probiotic, *Bacillus coagulans*, as a food ingredient. *Food and Chemical Toxicology*, *47*, 1231–1238.

Fujiya, M., Musch, M. W., Nakagawa, Y., Hu, S., Alverdy, J., Kohgo, Y., et al. (2007). The *Bacillus subtilis* quorum-sensing molecule CSF contributes to intestinal homeostasis via OCTN2, a host cell membrane transporter. *Cell Host & Microbe*, *1*, 299–308. https://doi.org/10.1016/j.chom.2007.05.004.

Ghelardi, E., Celandroni, F., Salvetti, S., Gueye, S. A., Lupetti, A., & Senesi, S. (2015). Survival and persistence of *Bacillus clausii* in the human gastrointestinal tract following oral administration as spore-based probiotic formulation. *Journal of Applied Microbiology*, *119*, 552–559.

Gibson, G. R., Probert, H. M., Van Loo, J., Rastall, R. A., & Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. *Nutrition Research Reviews*, *17*, 259–275.

Gyawali, R., Nwamaioha, N., Fiagbor, R., Zimmerman, T., Newman, R. H., & Ibrahim, S. A. (2019). The role of prebiotics in disease prevention and health promotion. *Dietary interventions in gastrointestinal diseases*. Amsterdam, The Netherlands: Elsevier. pp. 151–167. https://doi.org/10.1016/B978-0-12-814468-8.00012-0.

Hamdy, A. A., Elattal, N. A., Amin, M. A., Ali, A. E., Mansour, N. M., Awad, G. E., et al. (2018). *In vivo* assessment of possible probiotic properties of *Bacillus subtilis* and prebiotic properties of levan. *Biocatalysis and Agricultural Biotechnology*, *13*, 190–197.

Henriques, A. O., & Moran, C. P. (2007). Structure, assembly, and function of the spore surface layers. *Annual Review of Microbiology*, *61*, 555–588.

Hong, H. A., Duc, L. H., & Cutting, S. M. (2005). The use of bacterial spore formers as probiotics: Table 1. *FEMS Microbiology Reviews*, *29*, 813–835. https://doi.org/10.1016/j.femsre.2004.12.001.

Hong, H. A., Huang, J. -M., Khaneja, R., Hiep, L. V., Urdaci, M. C., & Cutting, S. M. (2008). The safety of *Bacillus subtilis* and *Bacillus indicus* as food probiotics. *Journal of Applied Microbiology*, *105*, 510–520. https://doi.org/10.1111/j.1365-2672.2008.03773.x.

Hoyles, Lesley, et al. (2012). Recognition of greater diversity of Bacillus species and related bacteria in human faeces. *Research in Microbiology*, *163*(1), 3–13. doi: 10.1016/j.resmic.2011.10.004.

Huang, J. M., et al. (2008). Immunostimulatory activity of Bacillus spores. *FEMS Immunology & Medical Microbiology*, *53*(2), 195–203. doi: 10.1111/j.1574-695X.2008.00415.x.

Hyronimus, B., Le Marrec, C., Sassi, A. H., & Deschamps, A. (2000). Acid and bile tolerance of spore-forming lactic acid bacteria. *International Journal of Food Microbiology*, *61*, 193–197.

Jafarpour, D., Shekarforoush, S. S., Ghaisari, H. R., Nazifi, S., Sajedianfard, J., & Eskandari, M. H. (2017). Protective effects of synbiotic diets of *Bacillus coagulans*, *Lactobacillus plantarum* and inulin against acute cadmium toxicity in rats. *BMC Complementary and Alternative Medicine*, *17*, 291. https://doi.org/10.1186/s12906-017-1803-3.

Joseph, B., Dhas, B., Hena, V., & Raj, J. (2013). Bacteriocin from *Bacillus subtilis* as a novel drug against diabetic foot ulcer bacterial pathogens. *Asian Pacific Journal of Tropical Biomedicine*, *3*, 942–946. https://doi.org/10.1016/S2221-1691(13)60183-5.

Kotb, E. (2015). Purification and partial characterization of serine fibrinolytic enzyme from *Bacillus megaterium* KSK-07 isolated from kishk, a traditional Egyptian fermented food. *Applied Biochemistry and Microbiology*, *51*, 34–43. https://doi.org/10.1134/S000368381501007X.

Kotiranta, A., Lounatmaa, K., & Haapasalo, M. (2000). Epidemiology and pathogenesis of Bacillus cereus infections. *Microbes and Infection*, *2*(2), 189–198.

Krumbeck, J. A., Walter, J., & Hutkins, R. W. (2018). Synbiotics for improved human health: Recent developments, challenges, and opportunities. *Annual Review of Food Science and Technology*, *9*, 451–479. https://doi.org/10.1146/annurev-food-030117-012757.

Lee, N.-K., Kim, W.-S., & Paik, H.-D. (2019). *Bacillus* strains as human probiotics: Characterization, safety, microbiome, and probiotic carrier. *Food Science and Biotechnology*, *28*(5), 1297–1305. doi: 10.1007/s10068-019-00691-9. 31695928.

Lee, N. -K., Son, S. -H., Jeon, E. B., Jung, G. H., Lee, J. -Y., & Paik, H. -D. (2015). The prophylactic effect of probiotic *Bacillus polyfermenticus* KU3 against cancer cells. *Journal of Functional Foods*, *14*, 513–518.

Lund, T., & Granum, P. E. (1997). Comparison of biological effect of the two different enterotoxin complexes isolated from three different strains of Bacillus cerous. *Microbiology*, *143*(10), 3329–3336.

Nicoleta, Maricica, M. (2019). Probiotic, prebiotic and synbiotic products in human health. In R. Lidia Solís-Oviedo, & Á. de la Cruz Pech-Canul (Eds.), *Frontiers and new trends in the science of fermented food and beverages*. London: IntechOpen. https://doi.org/10.5772/intechopen.81553.

Mannanov, R. N., & Sattarova, R. K. (2001). Antibiotics Produced by *Bacillus* bacteria. *Chemistry of Natural Compounds*, *37*, 117–123. https://doi.org/10.1023/A:1012314516354.

Markowiak, P, & Śliżewska, K (2017). Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients*, *9*, 1021. https://doi.org/10.3390/nu9091021.

Mazza, P. (1994). The use of Bacillus subtilis as an antidiarrhoeal microorganism. *Bollettino Chimico Farmaceutico*, *133*(1), 3–18. 8166962.

Minamida, K., Nishimura, M., Miwa, K., & Nishihira, J. (2015). Effects of dietary fiber with *Bacillus coagulans* lilac-01 on bowel movement and fecal properties of healthy volunteers with a tendency for constipation. *Bioscience, Biotechnology, and Biochemistry*, *79*, 300–306. https://doi.org/10.1080/09168451.2014.972331.

Moir, A. (2006). How do spores germinate? *Journal of Applied Microbiology*, *101*, 526–530. https://doi.org/10.1111/j.1365-2672.2006.02885.x.

Nicholson, W. L., Munakata, N., Horneck, G., Melosh, H. J., & Setlow, P. (2000). Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. *Microbiology and Molecular Biology Reviews*, *64*, 548–572. https://doi.org/10.1128/MMBR.64.3.548-572.2000.

Nista, E. C, et al. (2004). Bacillus clausii therapy to reduce side-effects of anti-Helicobacter pylori treatment: randomized, double-blind, placebo controlled trial. *Alimentary Pharmacology & Therapeutics*, *20*(10), 1181–1188. doi: 10.1111/j.1365-2036.2004.02274.x.

Nyangale, E. P., et al. (2014). Effect of prebiotics on the fecal microbiota of elderly volunteers after dietary supplementation of Bacillus coagulans GBI-30, 6086. *Anaerobe*, *30*, 75–81. doi: 10.1016/j.anaerobe.2014.09.002.

Pandey, K. R., Naik, S. R., & Vakil, B. V. (2015). Probiotics, prebiotics and synbiotics—A review. *Journal of Food Science and Technology*, *52*, 7577–7587. https://doi.org/10.1007/s13197-015-1921-1.

Pinchuk, I. V., Bressollier, P., Verneuil, B., Fenet, B., Sorokulova, I. B., Mégraud, F., et al. (2001). In vitro anti-*Helicobacter pylori* activity of the probiotic strain *Bacillus subtilis* 3 is due to secretion of antibiotics. *Antimicrobial Agents and Chemotherapy*, *45*, 3156–3161. https://doi.org/10.1128/AAC.45.11.3156-3161.2001.

Rhee, K. -J., Sethupathi, P., Driks, A., Lanning, D. K., & Knight, K. L. (2004). Role of commensal bacteria in development of gut-associated lymphoid tissues and preimmune antibody repertoire. *Journal of Immunology*, *172*, 1118–1124.

Ringø, E., & Song, S. K. (2016). Application of dietary supplements (synbiotics and probiotics in combination with plant products and β-glucans) in aquaculture. *Aquaculture Nutrition*, *22*, 4–24. https://doi.org/10.1111/anu.12349.

Ripert, G., Racedo, S. M., Elie, A. -M., Jacquot, C., Bressollier, P., & Urdaci, M. C. (2016). Secreted compounds of the probiotic *Bacillus clausii* strain O/C inhibit the cytotoxic effects induced by *Clostridium difficile* and *Bacillus cereus* toxins. *Antimicrobial Agents and Chemotherapy*, *60*, 3445–3454. https://doi.org/10.1128/AAC.02815-15.

Rogha, M., Esfahani, M. Z., & Zargarzadeh, A. H. (2014). The efficacy of a synbiotic containing *Bacillus coagulans* in treatment of irritable bowel syndrome: A randomized placebo-controlled trial. *Gastroenterology and Hepatology from Bed to Bench*, *7*, 156–163.

Sanders, M. E., Morelli, L., & Tompkins, T. A. (2003). Sporeformers as human probiotics: *Bacillus*, *Sporolactobacillus*, and *Brevibacillus*. *Comprehensive Reviews in Food Science and Food Safety*, *2*, 101–110.

Shinde, T., Perera, A. P., Vemuri, R., Gondalia, S. V., Beale, D. J., Karpe, A. V., et al. (2020). Synbiotic supplementation with prebiotic green banana resistant starch and probiotic *Bacillus coagulans* spores ameliorates gut inflammation in mouse model of inflammatory bowel diseases. *European Journal of Nutrition*, *59*(8), 3669–3689. doi: 10.1007/s00394-020-02200-9. 32067099.

Sorokulova, I. B., Pinchuk, I. V., Denayrolles, M., Osipova, I. G., Huang, J. M., Cutting, S. M., et al. (2008). The safety of two *Bacillus* probiotic strains for human use. *Digestive Diseases and Sciences*, *53*, 954–963.

Spielholz, C. (2011). Efficacy of a synbiotic chewable tablet in the prevention of antibiotic-associated diarrhea. *Health (N. Y.)*, *3*, 110.

Tam, N. K., et al. (2006). The intestinal life cycle of Bacillus subtilis and close relatives. *Journal of Bacteriology*, *188*(7), 2692–2700. doi: 10.1128/JB.188.7.2692-2700.2006.

Tidjiani Alou, M., Rathored, J., Khelaifia, S., Michelle, C., Brah, S., Diallo, B. A., et al. (2015). *Bacillus rubiinfantis* sp. nov. strain mt2T, a new bacterial species isolated from human gut. *New Microbes and New Infections*, *8*, 51–60. https://doi.org/10.1016/j.nmni.2015.09.008.

Urdaci, M. C., & Pinchuk, I. (2004). Antimicrobial activity of *Bacillus* probiotics. *Bacterial spore formers – Probiotics and emerging applications*. Norfolk, UK: Horizon Bioscience. pp. 171-182.

Vyas, U., & Ranganathan, N. (2012). Probiotics, prebiotics, and synbiotics: Gut and beyond. *Gastroenterology Research and Practice*, *2012*, 1–16. doi: 10.1155/2012/872716. 23049548.

Xianjun, B. L. D. X. L., & Fei, W. Y. Z. (2012). Effects of *Bacillus subtilis*-dahlin synbiotics on growth performance and humoral immunity in weaner piglets. *Chinese Journal of Animal Nutrition*, *2*.

Zhao, G., Shao, L., & Gong, J. (2015). Effects of *Bacillus subtilis*-acanthopanax polysaccharide synbiotics on the ileum organization structure and duodenum digestive enzymes of chicks. *China Feed*, *2015*, 12.

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Chapter 16

Probiotic Fortified Seaweed Silage as Feed Supplement in Marine Hatcheries

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1 Introduction

World fish production reached a maximum of 171 million tonnes in 2016, with aquaculture contributing 47% of the total production. Total fish output sales value was estimated at USD 362 billion, of which USD 232 billion was from aquaculture. Contribution of the capture fisheries production was relatively static since the late 1980s at about 80–90 million tonnes annually; aquaculture has been responsible for the continuing growth in the supply of fish for global fish consumption. In an FAO (2018) report, global capture fisheries production was 90.9 million tonnes, where fisheries in marine and inland waters provided 87.2% and 12.8%, respectively. Aquaculture continues to grow faster than other major food production, although it no longer enjoys the high annual growth rates of the 1980s and 1990s (11.3% and 10.0%). Average annual growth has declined to 5.8% during the period 2000–16, but double-digit growth still occurred in a small number of individual countries, particularly in Asia and Africa from 2006 to 2010. In 2016 the average annual increase in global food fish consumption (3.2%) outpaced population growth (1.6%) and exceeded that of meat from all terrestrial animals combined (2.8%). Fish also provided close to 3.2 billion people with 20% of per capita intake of animal protein. Global aquaculture production in 2016 included 80.0 million tonnes of food fish and 30.1 million tonnes of aquatic plants, as well as 37,900 t of nonfood products (FAO, 2018). The major aquaculture producers in 2016 were China, India, Indonesia, Vietnam, Bangladesh, Egypt, and Norway.

The 2030 Agenda for Sustainable Development Goals has set aims for the contribution conduct of fisheries particularly the aquaculture sector toward food security and nutrition. There is a need to regulate the sector's use of natural resources by ensuring sustainable development in economic, social, and environment, within the context of the FAO Code of Conduct for Responsible Fisheries (FAO, 1995). A major challenge to the implementation of the 2030 Agenda is the sustainability disparity between developed and developing countries due to increased economic interdependencies, coupled with limited management and governance capacity that exists in developing countries. In an effort to eliminate disparity while making progress toward the target for food security by the 2030, the global community needs to support developing nations to achieve their full aquaculture potential *via* improved hatchery management and innovative approach that will enable these countries to use their natural resources in enhancing growth of their aquaculture sector. Here, we will look at the practicality and importance of introducing probiotics in the form of seaweed silage (SS) as a replacement diet of microalgal feed for fish larvae and rotifers in aquaculture hatcheries.

2 Issues in aquaculture hatcheries

Present aquaculture practices offer potential to enhance the production of valuable species in the following four ways: (1) direct enhancement of fish biomass; (2) production and release of cultured juveniles in an effort to increase the spawning biomass; (3) sea ranching operations, where cultured juveniles are released to supplement fishery catch; and (4) farming of cultured juveniles in earthen ponds and sea pens (Battaglene, Seymour, Ramofafia, & Lane, 2002; Agudo, 2006; Purcell et al., 2013). A commonality among these various approaches is the requirement for successful hatchery management that produces healthy fish larvae and juvenile fish. Here, consistent disease-free hatchery production remains a major bottleneck

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00016-2
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FIGURE 16.1 Open-air "Green-Water" production facility for the production of green microalgae as food source for fish larvae and rotifer (feed for fish fingerlings) in an Asian aquaculture fish hatchery.

in most aquaculture hatcheries, and this is particularly true among the hatcheries in Asia and Pacific regions. Present fish production approaches in fresh-water and marine-fish hatcheries are dependent on the utilization of large quantities of live, cultured microalgae to feed larvae and juveniles (Duy, Pirozzi, & Southgate, 2015). Mass culture of quality microalga is labor intensive, resource demanding, and prone to opportunistic bacteria outbreak (Coutteau & Sorgeloos, 1992; Purcell et al., 2013). Alternative food sources for larvae, such as phototrophically grown *Isochrysis* sp., *Pavlova* sp., and *Thalassiosira weissglogii*, are highly concentrated microalgae that are commercially available and nutritionally consistent larval food source, but their continuous availability is often a problem due to high cost and delivery issues to hatcheries that are situated in remote locations (Duy, Francis, Pirozzi, & Southgate, 2016).

Efforts to enhance fish production and reduce mortality in hatchery and grow-out systems warrants highly concentrated quality microalgae supply as first feed for fish larvae and rotifers. Recent study has revealed that disease outbreaks and larvae mortality are mainly caused by the presence of opportunistic bacteria and fungus in culture system due to the use of "Green-Water" or open-tank-cultured microalgae (Palmer, Burke, Palmer, & Burke, 2007) (Fig. 16.1). Since microalgae are necessary to feed fish larvae and rotifers, it has become important that the quality of microalgae be regulated and free from opportunistic microbes. Situation is further compromised when the traditional flow through culture systems is also prone to the intrusion of disease-causing microbes. Therefore, present larvae and fish fingerling production method with the use of mass-cultured microalgae as feed for rotifer and fish larvae in most hatcheries causes high mortality due to the presence of opportunistic microbes that are often found growing in hatcheries (Dahle et al., 2020). Various researchers have shown that the inclusion of probiotics in culture water either directly or *via* feed-incorporated introduction in growth-out fish facilities can drastically improve fish health and reduce mortality (Cavalcante et al., 2020). Probiotics for aquatic usage are different from that for terrestrial animals and human due to the intricate relationship between aquatic animals and ambient environment. Probiotics used in aquaculture are live microbes that have a beneficial effect on the host by modifying the host-associated ambient microbial community, improve the use of feed, enhance its nutritional value, increase the host response towards disease, and improve the quality of its ambient environment. This is vital as the administration of probiotics in aquaculture will have an impact of the culture medium, even if it is administrated as feed formulation.

3 Use of probiotics in aquaculture

Mortality increases at the "first feeding" stage when Green-Water microalgae are introduced regardless if the fish larvae are feed on solely microalgae or rotifers. So, it is important to lower the mortality by reducing the load of opportunistic microbial load in culture water system, guts of fish larvae, and rotifers that are used to feed fish fingerlings. Recent findings have shown that the introduction of probiotics reduces the opportunistic bacterial load and improves the survival rate of fish in the grow-out phase. In this regard, use of probiotics in fish culture has centered on the use of a consortium of lactic acid bacteria (Ghosh et al., 2021). Probiotic administration in aquaculture is based on the application of a live microbial food supplements that are consumed with the aim to provide health benefit to the host by contributing to an improved microbial balance within the intestinal microbiota (Guo et al., 2020). Beside, probiotic also acts as biologically active component of single or mixed culture that improves the health of the host (Tan et al., 2019) and enhances disease resistance (Miao et al., 2017). Today, probiotics (beneficial bacteria) are well accepted and widely used in aquaculture (Tan et al., 2019), as

it is used as the means to improve the quality of aquatic environment and fish immunity, while they are still at larvae stage. Larvae start feeding even though the digestive tract is not fully developed and though the immune system is still incomplete (Giri, Sukumaran, & Oviya, 2013). Therefore introduction of probiotics at larval stage is critical to reduce mortality and increase quality fish for grow-out culture (Balcazar et al., 2008). Functional importance of probiotics in aquaculture has been well researched and reported in finfish, shellfish, and sandfish aquaculture industries (Tan, Sai, & Shao, 2019; Miao et al., 2017; Duy et al., 2016). However, most fish mortality occurs during the larvae and fingerling stages, and this could not be solved by the direct introduction of probiotics into the culture medium. Larvae and fish fingerling have to ingest and populate their gut with a healthy community of probiotics; therefore the focus should be in the replacement of microalgae that is often introduced to the larvae and rotifers. In most hatcheries, open-cultured microalgae (Green-Water) are feed directly to the larvae or are encapsulated in the rotifers as these are fed to the fish fingerlings. Either way this is done, it has been shown to cost high percentage of mortality in larvae and fish fingerlings. The alternative feed should be similar in size as microalgae, contains high amount of nutrition, and is attractive to the larvae or fish fingerlings. The solution is the production of probiotic-fortified SS that is in the form of a detritus packed with probiotics and fine chemicals that will play an important role as nutrition and attractant.

Probiotic-fortified seaweed silage produced *via* fermentation of seaweed powder results in the production of single cell detritus (SCD) macroalgae that is laden with a consortium of *Lactobacillus* and is an ideal alternative to eliminate occurrences of opportunistic microbes to ensure continuous supply of healthy feed for aquaculture sector. SS was first introduced by Uchida, Nakata, & Maeda (1997) with the use of *Undaria pinnatifida* and a consortium of probiotic micro-organisms. Quality of SS often varies with the type of seaweed used in the fermentation process since the dynamics of the fermentation is heavily influenced by the inherently available fine chemicals of the seaweed (Uchida et al., 1997; Uchida & Murata, 2002).

4 Seaweed probiotic fermentation

Seaweeds with good nutrition and mineral are ideal to be considered as novel feed resource either as direct feed or as starting material in a more complex bioprocess enhanced product development. But, due to their high fiber and low protein content, many animal feed produces are reluctant to incorporate seaweed as part of their formula in aquafeed production. Innovative approach introduced by Uchida et al. (1997) was to transform seaweed into a fermented product with the use of probiotics, where it enhanced the nutritive value of the silage by enriching protein, vitamin, minerals, essential amino acids, fatty acids, and organic acids and reduced the material size of the seaweed into SCD. At the same time, probiotic-fortified SS acts as a growth promoter, immune enhancer, and probiotics in cultivable organisms. The fermentation process on the other hand improves the nutritive value, digestibility and enhances attractiveness of the feed toward fish larvae and fish fingerlings (Uchida, 2003).

Since 1997, brown seaweed such as *Undaria*, has been used as biomaterial in probiotic fermentation to produce SS. Brown seaweed has very high content of phenolic and other terpenoids and often produces a chemically concentrated silage. Uchida, Numaguchi, and Murata (2004) proposed the development of marine silage (MS) for the following three main reasons: (1) SS facilitates the conversion of algal biomass resources into dietary material for aquaculture, (2) SS is acidic and long-lasting at room temperature (reduces storage costs), and (3) fermented silage have functional chemicals with bioactive potentials. SS is also much easier to prepare as compared to microalgae culture, besides it is a probiotic food supplement. SS was first prepared by Uchida et al. (1997) and team using *U. pinnatifida* (Uchida et al., 2004). Algal frond tissues are decomposed during the saccharification process and resulted in a product that has a size of 5–10 μm in diameter. Formation of protoplasmic and spheroplasmic detritus *via* microbial degradation of macroalgal fronds is called "single cell detritus" (SCD) (Uchida et al., 1997). This small-size SCD is analogous to dietary phytoplankton and was suggested that SCD is used as the replacement of microalgae in hatchery diet preparation. In 2004, *Undaria* MS was demonstrated to contribute to the growth of bivalve, *Pinctada martensii* in a grow-out culture experiment. In addition, SS prepared from *Ecklonia maxima* was also introduced as supplement in formulated fin-fish diet and fed to the red sea bream fries with iridovirus. In conclusion, that study showed that SS also had an important function in promoting fish survival against the pathogen (Uchida & Murata, 2002).

Similar approach was also adopted by a research group from Spain lead by Camacho, Salinas, Delgado, and Fuertes (2007), where they also carried out experiments on clam (*Ruditapes decussatus*) growth and disease resistance using SCD produced from brown seaweed. Finding of the research showed that SS can replace 80%–90% of the live phytoplankton content of the clam diet with similar growth rate or even surpassing those exhibited when fed with live phytoplankton diet. Camacho concluded that SS from *Laminaria saccharina* is an ideal complement to live phytoplankton for molluscs. In 2011, a group of India researchers were successful in adapting the technology that Uchida developed (Uchida et al., 2004)

to produce feed for shrimp larvae. Probiotic fermentation of green seaweed (*Ulva reticulata)* with a consortium of probiotics that comprises of lactic acid bacterium (*Lactobacillus plantarum*) and yeast (*Saccharomyces cerevisiae*) was successfully demonstrated to be of importance in shrimp aquaculture sector (Felix & Pradeepa, 2011). Important addition to the fermentation mixture was the inclusion of potato and soya powder as sugar and nitrogen sources.

Latest trend in the utilization of probiotic microorganisms in aquaculture involves a multidisciplinary approach incorporating aspects of microbiology, fermentation dynamics, seaweed biochemistry, and marine natural product chemistry. Here, powdered seaweed thallus are converted into easily digestable soluble fibers of unicellular sizes known as Single Cell Detritus (SCD), that are laden with probiotics. In the course of the fermentation, breakdown of algal fine chemicals, and production of peptides by the probiotic organisms further enhances the final product with nutrients and bioactive substances. Here, we will deliberate on the latest findings pertaining to the probiotic fortified fermentation dynamics of red algae, *Eucheuma denticulatum* Doty. The resulting product was evaluated to have high quality of fine chemicals and was able to reduce mortality in marine fish hatcheries.

5 Probiotic fortifies seaweed silage of *Eucheuma denticulatum* Doty

Red algae, *Kappaphycus alvarezii* Doty and *E. denticulatum* Doty, are widely cultivated for their phycocolloid, *kappa*- and *iota*-carrageenan, respectively (Fig. 16.2). These are fast-growing seaweed where their harvest cycles are only about 100–120 days, and cultivation of these species is an important industry in Indonesia, Philippines, Malaysia, Zanzibar, Solomon Island, and Micronesia (Fig. 16.3). Carrageenan produced by these seaweeds is of commercial importance in food, medical, and cosmetic industries. In addition to its polysaccharide, carrageenophytes are also being utilized in small and medium domestic enterprises as a raw material in the production of food items in countries like Indonesia, Philippines, and Malaysia. *E. denticulatum* was used as a raw material in the production of SS on the basis of the following three important reasons: (1) fast-growing seaweed (300%–400% growth rate, could be harvested within 3 months), (2) selling price is lower than *K. alvarezii* (better to use it for downstream product development), and (3) *iota*-carrageenan is a softer phycolloid with more sulfated functional groups (biological activities are comparatively higher). Farm-cultured carrageenophytes



FIGURE 16.2 Cultivation and harvest of carrageenophytes. (A) Floating seaweed culture line, (B) matured seaweed, (C) harvesting of seaweed, and (D) postharvest drying of seaweed.

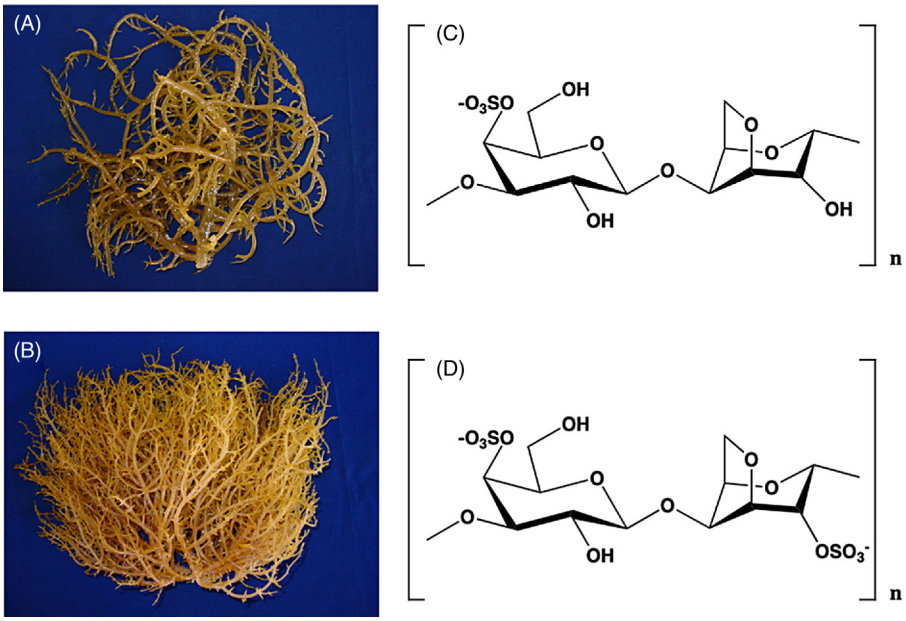


FIGURE 16.3 Commonly cultivated carrageenan producing seaweed species and its carrageenan type. (A) *Kappaphycus alvarezii* DOTY, (B) *Eucheuma denticulatum* DOTY, (C) *kappa*-carrageenan, and (4) *iota*-carrageenan.

are subjected to a postharvest desiccation process under shade to ensure minimum bleaching of its pigments and to avoid the destruction of its fine chemicals. Details of its postharvest handling practices and dynamics of carrageenophyte's fine chemicals have been described by Vairappan, Razalie, Elias, and Ramachandram (2014). Resulting desiccated seaweed was ground to produce seaweed powder of approximate particle size of 100 mm.

Conversion of seaweed powder into a silage was facilitated with the aid of cellulase enzyme for saccharification of seaweed bio-material and innoculation of a microbial mixture consisting of a consortium of *Lactobacillus* species and *S. cerevisiae* to initiate the fermentation process. Criteria for the selection of probiotic candidates for SS production were total maximum Colony Forming Unit (CFU) count of probiotic bacteria and nutritive quality of the final silage. Various combinations of Lactic Acid Bacteria (LAB) were initially experimented in an effort to determine the most suitable combination of microbes for the fermentation process, either individually or combination of several species. *Lactobacillus* utilized in this investigation consisted of (1) *Lactobacillus casei*, (2) *L. plantarum*, (3) *Lactobacillus fermentum*, and (4) *Lactobacillus sakei*. Fermentation formulation, consisted of a combination of 500 g seaweed powder, 40 g industrial cellulase (500 U g^{-1}), 100 mL lactic acid bacteria consortium (1.0 McFarland Index), and 100 mL *S. cerevisiae* (1.0 McFarland Index), was found to be the best mixture ratio for a 10 L volume of filtered water. Cellulase enzyme was introduced into the culture system with the seaweed powder, followed by the microbial mixtures, fermentation was carried out for a duration of 15 days.

Fig. 16.4 describes the growth dynamics of the respective *Lactobacillus* bacterial consortium in seaweed fermentation based on daily bacterial enumeration for 15 days of fermentation. As can be noticed, a total of seven different *Lactobacillus* combination cultures were used to identify the best species of lactobacillus for SS production for *E. denticulatum*. *L. casei* exhibited the highest total bacterial count and this was achieved within 3 days of culture period. Therefore *L. casei* was identified as the best species of Lactic Acid Bacterial (LAB) for SS. Next, batch fermentation was carried out with only *L. casei*, *S. cerevisiae*, and cellulase enzyme in the ratio described earlier to better understand the growth dynamics of microbes and pH fluctuation of the culture medium. Fermentation technique used for the production of SS does not require for its raw materials to be pre-autoclaved (seaweed powder and filter water) or sterile filtered (cellulase enzyme), while both the lactic acid bacteria and yeast cultures were pure cultures as these were cultured separately prior to fermentation mixture preparation. During this fermentation process, the following three different parameters were observed: (1) pH, (2) total Lactic Acid Bacteria (LAB) count, and (3) Miscellaneous Bacteria count. Fig. 16.5A describes the dynamics of culture medium pH values, while Fig. 16.5B shows the dynamics of total lactic acid bacteria count and Miscellaneous Bacteria count. pH during the fermentation process exhibited a drastic drop within the first 3 days to reach a value of 3.7, while the total Lactic Acid Bacteria (LAB) count was seen to show the same trend as the earlier fermentation process and the Miscellaneous Bacteria count showed a very low level of viability with gradual decline. This could be attributed to the drastic

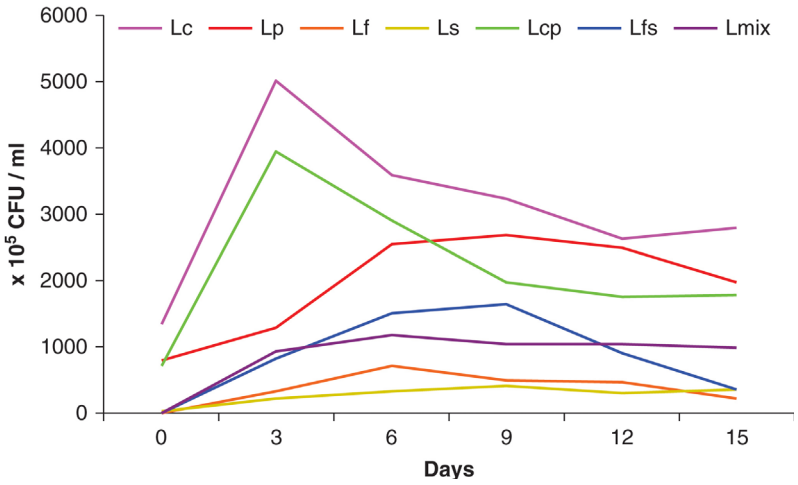


FIGURE 16.4 Growth dynamics and total colony forming unit (CFU) count of seven different *Lactobacillus* species in various combination cultures during fermentation of *Eucheuma denticulatum* for a duration of 15 days.

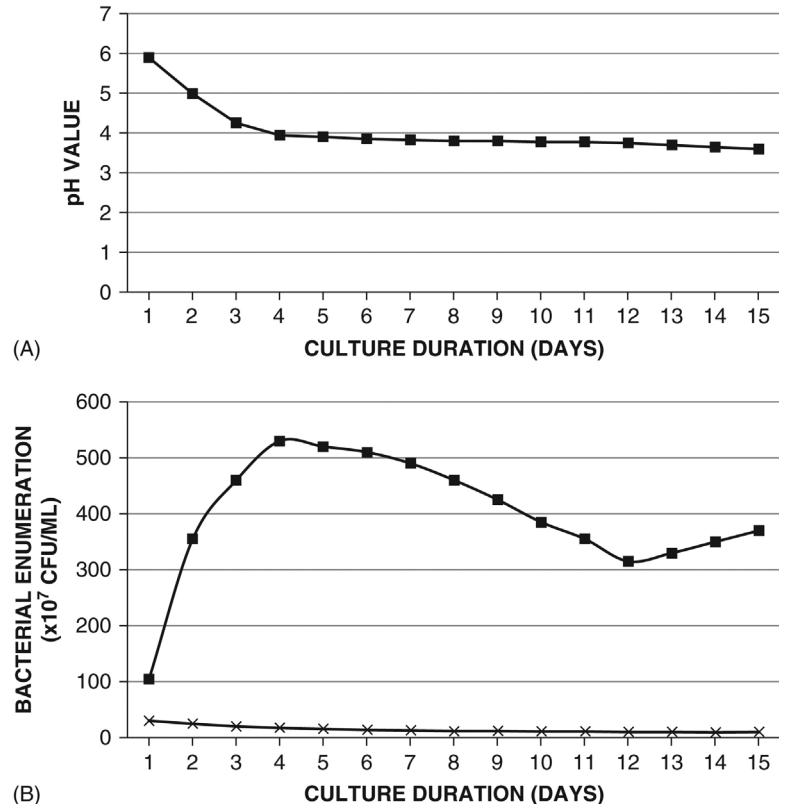


FIGURE 16.5 Dynamics and changes in pH (A), total *Lactobacillus* count and Miscellaneous Bacterial count (B) during the fermentation of *Eucheuma denticulatum* for a period of 15 days.

drop of culture pH value and high acidic levels that could not support the viability of these microbes. Resulting SS was ready for harvest in less than 7 days, SS was then analyzed for its particle size where the findings are shown in Fig. 16.6. Seaweed powder used during the start of the fermentation was in the range of 80–100 mm (Fig. 16.6A), due to the use of cellulase enzyme and reduction in the culture medium pH value, the particle size of the seaweed was reduced to 3–8 mm (Fig. 16.6B). These findings are somewhat better than the ones reported by Uchida for their SCD of *Undaria*, which was in the range of 5.8–11.5 mm (Uchida & Murata, 2002). Inoculation of LAB on Day-1 was 8.5×10^7 CFU mL^{-1}, and it increased drastically to 5.5×10^8 CFU mL^{-1} on Day-3. This is the maximum count. LAB count decreased gradually from

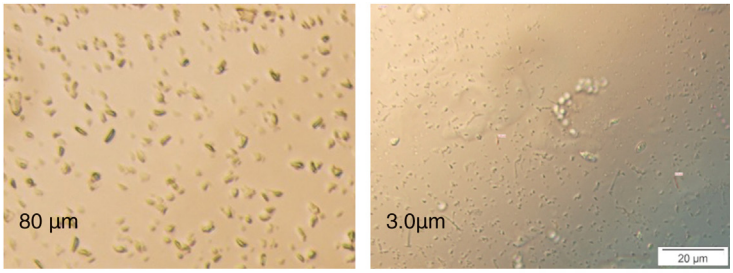


FIGURE 16.6 Particle size of seaweed thalli during the start of fermentation (A) and at the end of the fermentation process (B).

Day-3 to Day-12, which is 2.7×10^8 CFU mL^{-1}. However, there was a slight increment to 2.9×10^8 CFU mL^{-1} on the Day-15. The initial bacteria count for Miscellaneous Bacteria was 1.3×10^7 CFU mL^{-1}. However, these common bacterial growth decline from Day-1 to Day-15. The results showed that the pH dropped drastically while the growth of LAB was at its peak. The reduced pH could be attributed to the production of lactic acid by LAB. The accumulation of lactic acid makes the culture environment to become more acidic which led to the decrease in survival of common or Miscellaneous Bacteria.

6 Chemical characteristics of seaweed silage

The proximate compositions of the SS were evaluated, and the produced SS had a mean protein content of 18.23 ± 1.75%, 1.75 ± 0.05% lipid, 96.36 ± 3.28% moisture, and 32.40 ± 2.30% ash. The protein level in the silage is higher compared to the seaweed powder before fermentation, but this composition of protein is not sufficient for formulate feed for fish feeding. Most of the study on the protein requirement of fish larvae and fingerlings are based on the feeding efficiency and weight gain. Information from those study showed that the dietary protein requirement for fish fingerlings in local hatcheries are in the range of 25%–50%. However, lipid content in SS was too low compared to the basic requirement as recommended, 4%–7%. While the fermented SS contained 124 µg g^{-1} total fatty acid, represented by 54 µg g^{-1} saturated fatty acid (SFA), 54 µg g^{-1} monounsaturated fatty acid (MUFA), and 36 µg g^{-1} polyunsaturated fatty acid (PUFA). PUFAs present in fish, specially *n*-3 and *n*-6, are very important, which contributed to the reduction of incidence of cardiovascular diseases. Salini et al. (2015) proved that PUFA, especially the docosahexaenoic acid (DHA), has the ability in maintaining the structure and functional integrity of fish cells. The proximate composition of SS indicated that other supplements are needed in the final formulation of SS as good fish diet.

7 Seaweed silage as rotifer feed

Principle mortality factor in fish hatcheries is the outbreak of opportunistic bacteria during the use of "Green-Water" that contains microalgae. In an effort to reduce mortality, probiotic-fortified SS was introduced in the following two methods: (1) directly as feed and (2) as SS-encapsulated rotifers (*Brachionus* sp.), to fish larvae and fish fingerlings. Both these methods were experimentally executed in the hatchery and their data were recorded. It was apparent that the introduction of 3%–5% of SS for a given volume of fish culture medium was sufficient to support and yield healthy fish larvae, but it was difficult to maintain the water quality due to the presence of seaweed-derived soluble-fiber. Dissolve oxygen level was compromised in long-term utilization of the same culture medium. Therefore SS-encapsulation rotifer approach was found to be the best technique and it gave the highest reduction in fish mortality. It was much easier and practical to incorporate SS as feed for rotifers as the quality of water does not seem to deteriorate fast and there was no collapse in the cultured rotifer population. The SS was enumerated to be in the range of 2.0×10^7 (on Day-1) to 10.0×10^7 (on Day-5), with a particle in a 2–10 mm range per 20 mL dose. The overall pH of the fish tank was also kept in the range of 6.8–7.8 throughout the investigation duration. The conventional method of introducing microalgae via "Green-Water" or *Nannochloropsis* diet usually contained 1.0×10^8–2.5×10^8 cells per 20 mL with a cell size of 5–8 mm, we were able to match these concentration and cell sizes with the use of SS throughout these experiments. Fig. 16.7 shows the growth rate of rotifers when fed with SS for a duration of 5 days. A total of four different feeding load was experimented, 0.5%, 1.0%, 2.0%, and 4.0% of the culture volume. As shown in Fig. 16.7, rotifer growth rate (r) as calculated on the basis of studies of Park et al. (2001) was observed as 0.43, 0.51, 0.63, and 0.36, respectively, for 0.5%, 1.0%, 2.0%, and 4.0% feed load. Rotifers fed with 2.0% of SS also gave the highest percentage of rotifers with eggs in addition to best growth rate. The seaweed thalli were degraded with the use of cellulase enzyme and acid produced by the lactic acid bacteria during the fermentation process, this

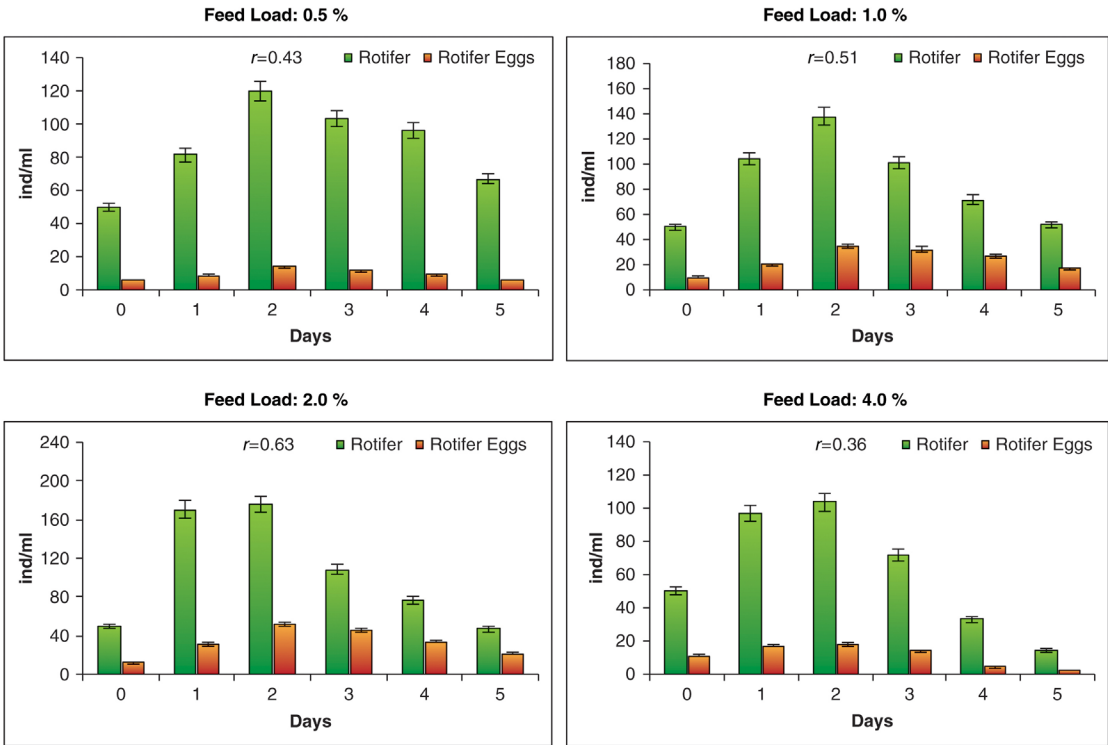


FIGURE 16.7 Growth profile of total rotifers and rotifers with eggs for the duration of 5 days when cultured with different feed load percentage of seaweed silage.

facilitated in the size reduction to about <10 mm in the final SS product. Nutritional profile of the final SS product upon fermentation was ideal as an alternative feed for the rotifers. However, in an effort to enhance the nutritional properties of the probiotic-fortified SS, we also conducted another set of experiment with the combination of SS and *Nannochloropsis* diet (1:1 ratio) (commercially available frozen microalgae). Fig. 16.8 describes the outcome of this experiments; it could be seen that the growth rates of rotifers were significantly much higher for the same feed load as with only SS. Rotifer growth rates (*r*) as calculated on the basis of studies of Park et al. (2001) were 0.84, 1.24, 1.46, and 1.0 for feed load of 0.5%, 1.0%, 2.0%, and 4.0%, respectively. The best rotifer growth and density of rotifers with eggs were found in the feed load of 2.0% within 2 days of feeding. Similar experiment with only *Nannochloropsis* at 2.0% feed load only gave a growth rate of less than 0.5 and a much lower density of rotifers with eggs. Based on these experiments, we were able to show that introduction of SS could increase growth rate of rotifers and a much higher rotifer culture could be attained when SS is used in combination with *Nannochloropsis*. Rotifers were successfully demonstrated as a vehicle of encapsulation of SS. The high density of rotifers with eggs also increased the nutritive value and attractiveness of the fish feed. Utilization of rotifers as fish feed was able to reduce the mortality to about 70%–80% in *Lates calcarifer* (Asian seabass) fish fingerlings.

8 Seaweed silage feed formulation

In addition to the direct and SS-encapsulated rotifer application of probiotic-fortified seaweed SCD, SS was also formulated into dry fish feed (<6% moisture) for 1–2 in. Asian seabass, *L. calcarifer.* Asian seabass is a well-utilized species in most hatcheries in Asian countries as it is a euryhaline fish with local and export potential. Hatchery practices involve larva production, nursery, and grow-out phases, have intensified to increase production of this species based on the demands of local and export market. It is well known fact that intensification is often accompanied by the increase in diseases and problems caused by viruses, bacteria, fungi, and other pathogens. It is a common practice to use antibiotics and chemotherapeutics to treat fish health problems, but as concerns of food safety and antibiotic-resistant bacteria rise, it has become necessary to consider non-chemotherapeutic methods (Cabello, 2006; De et al., 2014).

Produced SS was incorporated with additional ingredients to enhance the essential nutritional properties. Experimental diets were prepared based on the protein (45%) and lipid (10%) requirements of Asian seabass as per the method of Chiu et al. (2015). The ingredients were ground up in a hammer mill to pass through a 60-mesh screen. Diets were prepared by mixing ingredients with 4.5% fish oil, and moisture was maintained at 35% that results a stiff dough. Dough was passed

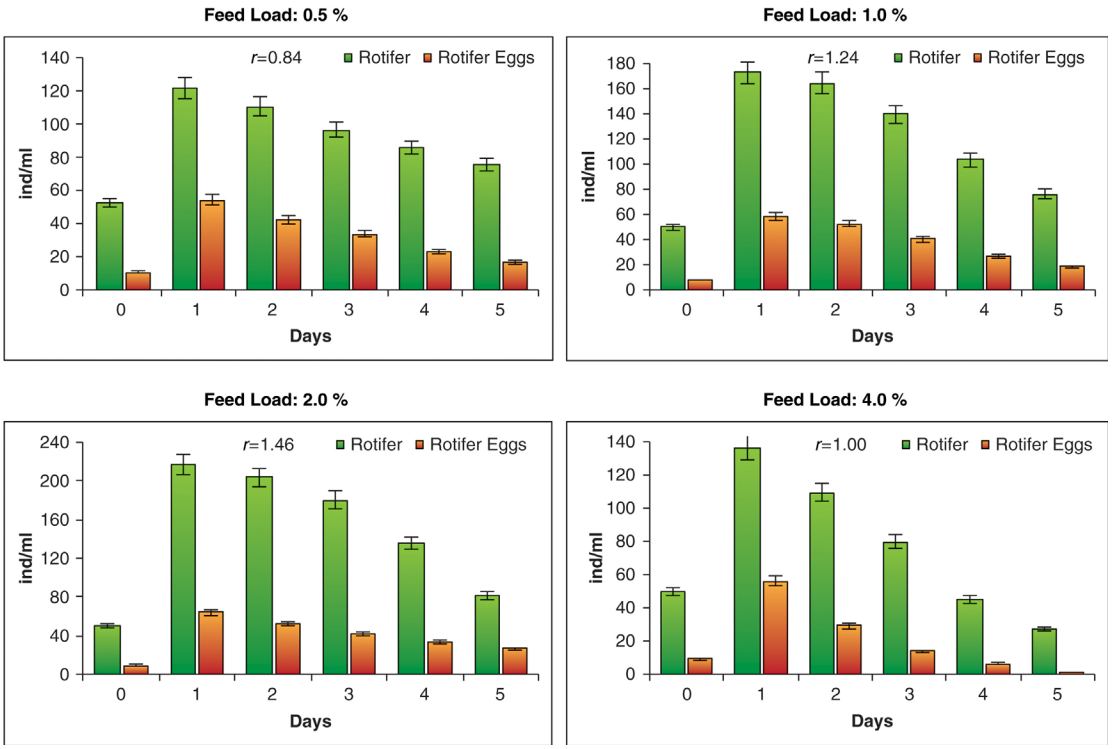


FIGURE 16.8 Growth profile of total rotifers and rotifers with eggs for the duration of 5 days when cultured with different feed load percentage of combination of seaweed silage and *Nannochloropsis* (1:1).

through a food grinder and resulting pellets were cut to desired particle size (<1 cm) and dried in drying cabinets at 80°C to a moisture level <6%. Proximate compositions of the diets were determined following methods of the Association of Official Analytical Chemists (AOAC, 1984). Seabass juveniles were bought from a private farm (80 fingerlings, average weight of 2.25 ± 0.05 g) during acclimation fish were kept at polyvinyl tanks (6 × 2 × 1 m) with continuous water flow and aeration at 24°C. Fish were fed with commercial diet at a ratio of 5% of their body weight once daily until experiments began. Fish feces and uneaten portions of the feed were removed with a siphon once daily prior to feeding. Fish weights were measured at the start of the experiment and at 2-week intervals until the end of the 8-weeks experiment. Water temperature, dissolved oxygen, and pH were respectively monitored daily using a YSI multiprobe meter.

A total of three different diet formulas were used in these experiments, two were prepared (Pellet A and B) (Fig. 16.9) and control was the commercial pellet. Both the prepared pellets were different in their composition and were used in feeding trails with commercial pellets as control. Pellet A was formulated with probiotic-fortified SS powder, while Pellet B was formulated with wheat flour as a substitution of MS. The nutritive value of the formulation was enhanced with the addition of other supplements such as krill, binder, vegetable fat, and cod liver oil. Pellet A had a light brownish, smooth textured with a distinctive seaweed-krill odor, while Pellet B had a sienna color, rough textured with a stronger krill odor as compared to PA. Commercial pellet was in saddle color, drier pellet with a fishy odor, and rough texture. The protein and lipid contents were kept to the level warranted by the requirements of Asian seabass per the suggestion of Chiu, Shiu, Wu, Lin, and Liu (2015). Both Pellet A and B consist of highest amount of protein and lipid content, 45%–47%, and 12%–14%, respectively. Locally available commercial feed was used as a control, its protein and lipid levels were as required for this investigation.

The growth of fish fed with three different experimental diets showed clear differences. The growth was recorded according to the weight gain of fish within 56 days. Fish fed with Pellet A indicated best growth, followed by commercial pellet and Pellet B. Fish fed with probiotic-fortified feed (PA) had a final weight gain of 48.97 ± 1.64 g at the Day-56, whereas fish fed with commercial pellet and Pellet B had a final weight gain of 32.36 ± 1.10 g and 38.16 ± 1.08 g, respectively. Survival percentage throughout this investigation was 95%–100%. The improvement of fish growth fed with fortified probiotic fish feed may due to the improvement in nutrient digestibility. Feed efficiency was accounted as 1.25, 0.98, and 0.90 for Pellet A, commercial pellet, and Pellet B, respectively. Several researchers reported that feeding diets supplemented with probiotic may improve appetite, health status, growth performances and reduce the mortality rate of farmed fish such

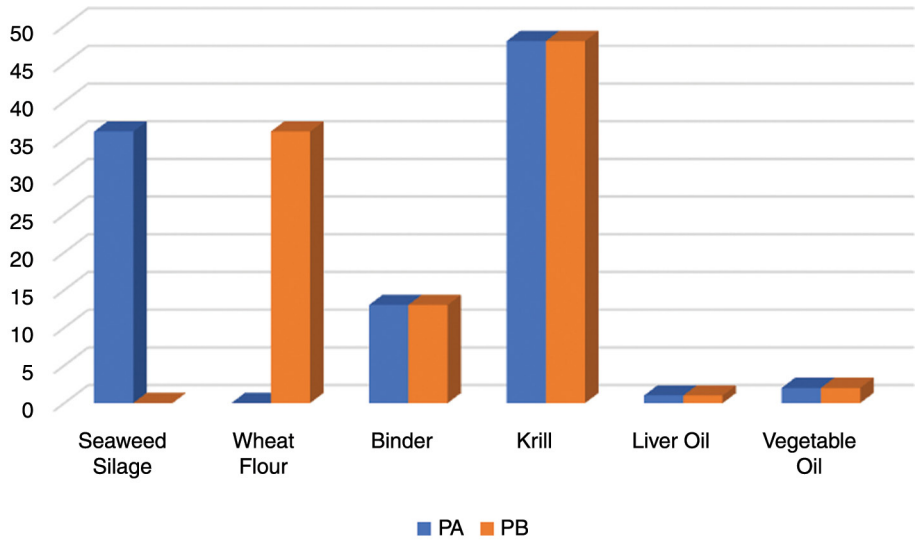


FIGURE 16.9 Formulation of fish feed prepared at the laboratory. Pellet A with the addition of seaweed silage, Pellet B with the addition of wheat flour to replace seaweed silage (Pellet A: seaweed silage—36%, binder—13%, krill—48%, vegetable oil—2%, liver oil—1%; Pellet B: wheat flour—36%, binder—13%, krill–48%, vegetable oil–2%, liver oil–1%).

as sea bass and rainbow trout (Salini et al., 2015; Burr, Gatlin, & Ricke, 2007). Recent findings of Lin, Shiu, Chiu, Huang, and Liu (2017) demonstrated that direct addition of probiotic mixture into the culture medium of the Asian seabass at the concentration of 10^9 CFU (kg diet^{-1}) could provide adequate probiotic efficiency for Asian seabass.

It has been reported by several researchers that some species of *Lactobacillus* such as *L. bulgaricus*, *L. acidophilus*, *L. sporogenes*, *L. casei*, and *L. plantarum* are effectively used as probiotics in fish feed formulations (Ringo & Gatesoupe, 1998). According to Essa, Mabrouk, Mohamed, and Michael (2011), there are significant improvements in the growth and digestive enzymes (amylase, protease, and lipase) activities due to the administration of probiotics in finfish diet as compared to control diet which had no probiotic supplement. Data obtained using SS of *E. denticulatum* also prove that probiotic-fortified SS contributes to fast growth and higher feed conversion efficiency in fish fingerlings of *L. calcarifer*. Beside lactic acid bacteria, yeast has also been attributed to have a positive contribution in fish growth. It has been used either as a live or processed feed ingredients in fish diet to improve the growth performance of fish. Essa et al. (2011), demonstrated that finfish fed with diets supplemented with *S. cerevisiae* showed a significant improvement in growth rate compared to the control diet without it. Similar finding was also reported with fish species such as sea bass, *Dicentrarchus labrax* (Tovar et al., 2002) and Japanese flounder, *Paralichthys olivaceus* (Harikrishnan, Balasundaram, & Heo, 2010). This might be due to the ability of yeast to secrete polyamines which is the essential growth factors and immunostimulants as described by Yu et al. (2014).

9 Conclusion

Importance of probiotics in aquaculture is well understood and widely accepted as a best approach to enhance fish growth and reduce disease incidence. Direct administration of probiotics into the culture medium seems to be the preferred means of utilization. However, this approach involves excessive use of lactic acid bacteria, and pH of the culture medium could be compromised. Therefore, the utilized consortium of probiotic strains should be safe for both the aquatic host and human, besides it should also be adaptable, able to survive the intestinal tract of the aquatic host, and survive in the aquatic environment without any mutation or loss of functional proteins. Most hatcheries are still using microalgae either cultured as Green-Water or purchased as high concentrated microalgal diet to feed fish larvae or rotifers. This practice has its drawback in terms of cost and possible opportunistic microbial outbreak that could result in mass mortality of fish larvae. Therefore, introduction of probiotics from larval stage is an important step to increase fish production in hatcheries. The production of SS using *E. denticulatum* is one way to address the high mortality due to the use of "Green-Water" as a source of microalgae in most Asian hatcheries. As fish larva production and survival will determine the success of any aquaculture hatchery, incorporation of probiotics from the larval stage of fish development will enhance their survival and growth rates. Besides, production of SS is much cheaper, easier and reduces the hatchery space needed for the conventional "Green-Water," and it also drastically reduces the outbreak of opportunistic bacteria that could induce high percentage of mortality.

Acknowledgments

The author would like to express his gratitude to Ms. Sangeetha Priya Anandan, and Ms. Tan Hsin Lin for their assistance during the preparation of seaweed silage and animal experiments. This research was funded by the Ministry of Agriculture (Malaysia) through research grants: (1) Exploration of selected Sabah edible seaweeds as potential source of probiotics (ERGS0039-STWN-1-2013), and (2) A pre-commercial marine fish hatchery model for strategic development of Malaysian aquaculture (Techno-Fund MOA (Aqua Vision (M) Sdn Bhd)) (TF0310M158).

References

Agudo, N. (2006). *Sandfish hatchery techniques*. Canberra, Australia: Noumea, Australian Centre for International Agricultural Research (ACIAR), the Secretariat of the Pacific Community (SPC) and the World Fish Center. 44. 978-982-00-0175-6.

AOAC. (1984). *Official methods of analysis of the Association of Official Analytical Chemists* (14th ed., pp. 240–290). Arlington, VA: Association of Official Analytical Chemists.

Balcazar, J. L., Blas, I., Ruiz-Zarzuele, I., Cunningham, D., Vendrell, D., & Muzquiz, J. L. (2008). The role of probiotics in aquaculture. *Veterinary Microbiology*, *114*(3), 173–186.

Battaglene, S. C., Seymour, J. E., Ramofafia, C., & Lane, I. (2002). Spawning induction of three tropical sea cucumbers, *Holothuria scabra*, *H. fuscogilva* and *Actinopyga mauritiana*. *Aquaculture*, *207*, 29–47.

Burr, G., Gatlin, D., & Ricke, S. (2007). Microbial ecology of the gastrointestinal tract of fish and the potential application of prebiotics and probiotics in fin fish aquaculture. *Journal of the World Aquaculture Society*, *36*, 425–436.

Cabello, C. F. (2006). Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. *Environmental Microbiology*, *8*, 1137–1144.

Camacho, A. P., Salinas, J. M., Delgado, M., & Fuertes, C. (2007). Use of single cell detritus (SCD) produced from *Laminaria saccharina* in the feeding of the clam *Ruditapes decussatus* (Linnaeus1758). *Aquaculture*, *266*(1–4), 211–218.

Cavalcante, R. B., Telli, G. S., Tachibana, L., Dias, D. C., Oshiro, E., Natori, M. M., et al. (2020). Probiotics, prebiotics and synbiotics for Nile tilapia: Growth performance and protection against *Aeromonas hydrophila* infection. *Aquaculture Reports*, *17*, 100343.

Chiu, S. T., Shiu, Y. L., Wu, T. M., Lin, Y. S., & Liu, C. H. (2015). Improvement in non-specific immunity and disease resistance of barramundi, *Lates calcarifer* (Bloch), by diets containing *Daphnia similis* meal. *Fish and Shellfish Immunology*, *44*, 172–179.

Coutteau, P., & Sorgeloos, P. (1992). The use of algal substitutes and the requirement for live algae in the hatchery and nursery rearing of bivalve molluscs: An international survey. *Journal of Shellfish Research*, *11*, 267–476.

Dahle, S. W., Bakke, I., Birkeland, M., Nordoy, K., Dalum, A. S., & Attramadal, K. J. K. (2020). Production of lumpfish (*Cyclopterus lumpus* L.) in RAS with distinct water treatments: Effects on fish survival, growth, gill health and microbial communities in rearing water and biofilm. *Aquaculture*, *522*, 735097.

De, B. C., Meena, D. K., Behera, B. K., Das, P., Mohapatra, P. K. D., & Sharma, A. P. (2014). Probiotics in fish and shellfish culture: Immunomodulatory and eco-physiological responses. *Fish Physiology and Biochemistry*, *40*, 921–971.

Duy, N. D. Q., Pirozzi, I., & Southgate, P. C. (2015). Ingestion of live micro-algae and micro-algae concentrates by sandfish, *Holothuria scabra*, larvae. *Aquaculture*, *448*, 256–261.

Duy, N. D. Q., Francis, D. S., Pirozzi, I., & Southgate, P. C. (2016). Use of micro-algae concentrates for hatchery culture of sandfish, *Holothuria scabra*. *Aquaculture*, *464*, 145–152.

Essa, M. A., Mabrouk, H. A., Mohamed, R. A., & Michael, F. R. (2011). Evaluating different additive levels of yeast, *Saccharomyces cerevisiae*, on the growth and production performances of a hybrid of two populations of Egyptian African catfish, *Clarias gariepinus*. *Aquaculture*, *320*, 137–141.

Food and Agriculture Organization of the United Nations. (1995). *The state of food and agriculture: Agriculture trade entering a new era?* Rome: Food and Agriculture Organization of the United Nations. 301. 92-5-103700-0.

Food and Agriculture Organization of the United Nations. (2018). *The state of world fisheries and aquaculture: Meeting the sustainable development goals*. Rome: Food and Agriculture Organization of the United Nations. 210. 978-92-5-130562-1.

Felix, S., & Pradeepa, P. (2011). Seaweed (*Ulva reticulata*) based fermented marine silage feed preparation under controlled conditions for *Penaeus monodon* larval development. *Journal of Marine Science: Research and Development*, *1*(1), 1–3.

Giri, S. S., Sukumaran, V., & Oviya, M. (2013). Potential probiotic *Lactobacillus plantarum* VSG3 improves the growth, immunity, and disease resistance of tropical freshwater fish, *Labeo rohita*. *Fish & Shellfish Immunology*, *34*(2), 660–666.

Ghosh, K., Mukherjee, A., Dutta, D., Banerjee, S., Brienes, E. M., Hareide, E., et al. (2021). Endosymbiotic pathogen-inhibitory gut bacteria in three Indian Major Carps under polyculture system: A step toward making a probiotics consortium. *Aquaculture and Fisheries*. online.

Guo, G., Li, C., Xia, B., Jiang, S., Zhou, S., Men, X., et al. (2020). The efficacy of lactic acid bacteria usage in turbot *Scophthalmus maximus* on intestinal microbiota and expression of the immune related genes. *Fish & Shellfish Immunology*, *100*, 90–97.

Harikrishnan, R., Balasundaram, C., & Heo, M. S. (2010). Effect of probiotics enriched diet on *Paralichthys olivaceus* infected with lymphocystis disease virus (LCDV). *Fish & Shellfish Immunology*, *29*, 868–874.

Lin, H. L., Shiu, Y. L., Chiu, C. S., Huang, S. L., & Liu, C. H. (2017). Screening probiotic candidates for a mixture of probiotics to enhance the growth performance, immunity and disease resistance of Asian seabass. *Lates calcarifer* (Bloch), against *Aeromonas hydrophila*. *Fish & Shellfish Immunology*, *60*, 474–482.

Miao, S., Zhu, J., Zhao, C., Sun, L., Zhang, X., & Chen, G. (2017). Effects of C/N ratio control combined with probiotics on the immune response, disease resistance, intestinal microbiota and morphology of giant freshwater prawan (*Macrobrachium rosenbergii*). *Aquaculture*, *476*, 125–133.

Palmer, P. J., Burke, M. J., Palmer, C. J., & Burke, J. B. (2007). Developments in controlled green-water larval culture technologies for estuarine fishes in Queensland Australia and elsewhere. *Aquaculture*, *272*(1–2), 1–21.

Park, H. G., Lee, K. W., Cho, S. H., Kim, H. S., Jung, M. M., & Kim, H. S. (2001). High density culture of freshwater rotifer, *Brachionus calyciflorus*. *Hydrobiologia*, *446–447*, 369–374.

Purcell, S. W., Mercier, A., Conand, C., Hamel, J. F., Toral-Granda, M. V., Lovatelli, A., et al. (2013). Sea cucumber fisheries: Global analysis of stocks, management measures and drivers of overfishing. *Fish and Fisheries*, *14*, 34–39.

Ringo, E., & Gatesoupe, F. J. (1998). Lactic acid bacteria in fish: A review. *Aquaculture*, *160*, 177–203.

Salini, M., Irvin, S., Bourne, N., Blyth, D., Cheers, S., Habilay, N., et al. (2015). Marginal efficiencies of long chain-polyunsaturated fatty acid used by barramundi (*Lates calcarifer*) when fed diets with varying blends of fish oil and poultry fat. *Aquaculture*, *449*, 48–57.

Tan, H. Y., Sai, W. C., & Shao, Y. H. (2019). Improvements in the growth performance, immunity, disease resistance, and gut microbiota by the probiotic *Rummeliibacillus stabekisii* in Nile tilapia (*Oreochromis miloticus*). *Fish & Shellfish Immunology*, *92*, 265–275.

Tovar, D., Zambonino, J., Cahu, C., Gatesoupe, F. J., Vazquez-Juarez, R., & Lesel, R. (2002). Effect of live yeast incorporation in compound diet on digestive enzyme activity in sea bass (*Dicentrarchus labrax*) larvae. *Aquaculture*, *204*, 113–123.

Uchida, M., Nakata, K., & Maeda, M. (1997). Introduction of detrital food webs into an aquaculture system by supplying single cell algal detritus produced from *Laminaria japonica* as a hatchery diet for *Anemia nauplii*. *Aquaculture*, *154*(2), 125–137.

Uchida, M., & Murata, M. (2002). Fermentative preparation of single cell detritus from seaweed, *Undaria pinnatifida*, suitable as a replacement hatchery diet for unicellular algae. *Aquaculture*, *207*(3–4), 345–357.

Uchida, M (2003). Use of fermented seaweed as a hatchery diet. *Aqua Feed International*, *2*, 15–17.

Uchida, M., Numaguchi, K., & Murata, M. (2004). Mass preparation of marine silage from *Undaria pinnatifida* and its dietary effect for young pearl oysters. *Fisheries Science*, *70*, 457–463.

Vairappan, C. S., Razalie, R., Elias, U. M., & Ramachandram, T. (2014). Effects of improved post-harvest handling on the chemical constituents and quality of carrageenan in red alga, *Kappaphycus alvarezii* Doty. *Journal of Applied Phycology*, *26*, 909–916.

Yu, H. H., Han, F., Xue, M., Wang, J., Tacon, P., Zheng, Y. H., et al. (2014). Efficacy and tolerance of yeast cell wall as an immunostimulant in the diet of Japanese seabass (*Lateolabrax japonicus*). *Aquaculture*, *432*, 217–224.

Chapter 17

Secondary Metabolites From Probiotic Metabolism

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1 Probiotics

Probiotics are live microorganisms, which when ingested in adequate amounts produce a range of beneficial effects on the health of the host (FAO/WHO, 2006). Probiotics have different biochemical mechanisms to maintain and promote health that include better adhesion to intestinal cells and inhibition of pathogens in these places, improvement of epithelial barrier, regulation of the immune function, and production of antibacterial substances, as well as, postbiotics (Lin et al., 2020). The most common probiotics belong to the genera *Lactobacillus*, which is classified as lactic acid bacteria (LAB), and *Bifidobacterium* (Georgieva, Peikova, Andonova, & Zlatkov, 2014). Within *Lactobacillus* genera, *Lactococcus*, *Enterococcus*, *Streptococcus*, and *Leuconostoc* are also classified as probiotics, as well as some fungi and yeast of genera *Aspergillus* and *Saccharomyces* genera (Amara & Shibl, 2015; Kechagia et al., 2013; Diez-Gutiérrez et al., 2020).

The microorganisms that are used as probiotics are recognized as safe or GRAS, and this safety status may be based either on a history of safe use in food prior to 1958 or on scientific procedures that require the same quantity and quality of evidence, as would be required for a food additive regulation (FDA, 2018). Therefore the term "probiotic" has been related to bacteria with beneficial effects for human health and to which a number of requirements are demanded (Lin et al., 2019), as can see in Table 17.1 (Lin et al., 2020).

Probiotics must be able to survive during their passage through the human gastrointestinal tract (GIT) and subsequently colonize the intestine. In addition, it is necessary that they reach the intestine as viable microbiota and in sufficient amount of approximately 10^7 CFU (Chávarri et al., 2010) in order to provide health benefits. Therefore probiotics must be resistant to the acidic conditions of the stomach and the high concentration of bile acids present in the small intestine (FDA, 2018; Kechagia et al., 2013). Angmo, Kumari, Savitri, & Bhalla, 2016 demonstrated that *Lactobacillus* was able to resist low pH conditions due to the presence of F0F1-ATPase activity (Angmo, Kumari, Savitri, & Bhalla, 2016; Behbahani, Noshad, & Falah, 2019) and that the different susceptibility of LAB to bile acids is due to their hydrolase activity (Angmo, Kumari, Savitri, & Bhalla, 2016; Wang et al., 2016a). In addition, LAB are tolerant to the osmotic pressure necessary for probiotic strains to survive in some foods such as cucumber (Lin et al., 2020). Likewise, Lin et al., 2020 confirmed that probiotic strains were capable of surviving high concentrations of bile acids (0.15%—1.10%), at low pH (2–4) and high osmotic pressures (2%–8%) (Winkelströter, Fabrício, Elaine, & Martinis, 2015; Park & Lim, 2015).

On the other hand, the ability of probiotics to adhere to epithelial cells produces beneficial effects in the intestine. Cell adhesion properties are considered to be correlated to aggregation, coaggregation, and hydrophobicity. In other words, the aggregation and coaggregation properties are important for the adhesion of probiotic strains, since they can direct bacterial adhesion to GIT (autoaggregation) and prevent colonization by pathogenic bacteria (coaggregation) (Armas, Camperio, & Marianelli, 2017). Hydrophobicity is one of the most important factors influencing the strength of bacterial adhesion. Therefore these characteristics allow probiotics to inhibit intestinal adhesion and colonization of pathogenic strains (Behbahani, Noshad, & Falah, 2019) (Table 17.1).

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00017-4
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TABLE 17.1 Requirements demanded for probiotics.

1. Generally Recognized as Safe (GRAS) at the strain level by the United States Food and Drug Administration (FDA) or as qualified presumption of safety (QPS) at the species level by the European Food Safety Authority (EFSA).
2. Obtained from breast milk, gut microbiota, and fermented foods.
3. Long used in food.
4. Proven to be safe, as a food or supplement.
5. A probiotic agent must show nonpathogenic properties.
6. Ability to survive in the digestive tract.
7. Colonization of the intestinal tract.
8. Production of antimicrobial substances.
9. Mainly *Lactobacillus* spp. and *Bifidobacterium* spp.; other bacteria such as *Lactococcus* spp., *Enterococcus* spp., *Streptococcus* spp., *Leuconostoc* spp.; and fungi and yeasts of the genus *Aspergillus* and *Saccharomyces cerevisiae*.

TABLE 17.2 Main functions of probiotics.

1. Produce beneficial metabolites (short-chain fatty acids, bacteriocins, reuterin, linoleic acid, and secondary bile acids).
2. Produce vitamin K and B vitamins [thiamin (B1), riboflavin (B2), pantothenic acid (B5), pyridoxine (B6), biotin (B7), folates (B9), cobalamin (B12)].
3. Produce beneficial proteins/peptides (optimize IgA production, enhance antimicrobial peptides production).
4. Reducing pathogenic toxins.
5. Increase intestinal cell activity and integrity of the epithelial layer.
6. Regulate the immune system and improve the antioxidative system.

Nowadays, one of the important characteristics of probiotics is the safety for human without harboring acquired and transferable antibiotic resistance (Zommiti, Nathalie, Jeannette, & Mounir, 2017). Some probiotic strains with intrinsic antibiotic resistance could be available for restoring the intestinal microbiota after an antibiotic treatment.

In recent decades, researchers have observed that probiotics produce compounds called postbiotics that have a beneficial effect on gut microbiota. Some of these compounds are short-chain fatty acids (SCFAs) that can reduce proinflammatory immune activity (Azad, Kalam, Sarker, Li, & Yin, 2018) as well as improve the integrity of the intestinal epithelial barrier (Park & Lim, 2015; Tulumoğlu, Halil, & Şimşek, 2014), optimize IgA production, modulate homeostatic bile acids production, and increase the production of antimicrobial peptides to prevent pathogen infections (Table 17.2).

Furthermore, probiotics can modulate the intestinal microbiota and generate antioxidant and anticancer compounds that block the synthesis of harmful enzymes in the gut (Molska & Reguła, 2019). Therefore these beneficial microorganisms can act systematically in nutrition, metabolism, physiology, and immunity and assist in the prevention of diseases (Lin et al., 2020).

To date, a large number of scientific articles have been published confirming that probiotics can produce beneficial effects in various gastrointestinal disorders, cardiovascular, and nervous system diseases, among others (Gomi et al., 2018).

2 Postbiotics

Generally, the metabolism is focused on the use of different nutrients that are transformed by biochemical reactions into precursors, known as metabolites, useful for the correct performance of microorganisms (Madigan, Bender, Buckley, & Sattley, 2019). Independently of the microbial species, the primary metabolism is based on the use of nutrients to stimulate cell proliferation and, thus, biomass synthesis (Chubukov, Gerosa, Kochanowski, & Uwe, 2014). Hence, the metabolites involved in primary metabolism are considered the main molecular skeleton of microorganisms (Thirumurugan, Alagappan Cholarajan, Suresh Raja, & Ramasamy, 2018).

By contrast, secondary metabolism plays a completely different role, since it is activated during the late growth phase and the metabolites produced are not essential for basic cell maintenance (Ruiz, Chávez, Forero, & García-Huante, 2010). However, these compounds can act as a defensive line against other organisms, behave as signaling molecules, enhance the transport of other compounds, or serve as bioactive complexes (Marinelli & Marcone, 2011).

According to Thirumurugan, Alagappan Cholarajan, Suresh Raja, & Ramasamy, 2018, more than 2,140,000 secondary metabolites have been described in the scientific literature. In most cases, secondary metabolites are synthesized by plants, followed by bacteria, fungi, and marine organisms such as corals, tunicates, or sponges (Thirumurugan, Alagappan

Cholarajan, Suresh Raja, & Ramasamy, 2018). In the case of microbial metabolites, a wide medical interest has been observed due to their potential therapeutic effect. For instance, several well-known microorganisms such as *Actinomycetes*, *Bacilli*, or probiotic bacteria, such as *Lactobacillus*, *Lactococcus*, or *Bifidobacterium*, can biosynthesize immune suppressants, chemotherapeutic, or antimicrobial compounds useful for human treatments (Craney, Salman, & Nodwell, 2013; Kholia, 2017).

Probiotic secondary metabolites are gaining interest due to the potential beneficial effect they could have in the pharmaceutical and food field (Ruiz, Chávez, Forero, & García-Huante, 2010). Recently, the bioactive functional metabolites from probiotics have been defined as postbiotics (Tsilingiri & Rescigno, 2013). The postbiotic term involves the metabolites or bacteria-free compounds released during the probiotic metabolism that could have a direct or indirect effect in the health of the host (Foo, Loh, Abdul Mutalib, & Abdul Rahim, 2019). Rad, Maleki, Kafil, & Abbasi, 2021 added that postbiotics are also considered those with novel chemical structures, nontoxic effects, and easily absorbed, metabolized, and excreted compounds. The production of this kind of compound is directly linked to specific physicochemical conditions and, therefore, it is possible to focus the metabolism on their synthesis. However, these conditions can also stimulate the synthesis of unknown postbiotics, since their effectiveness have been proven without identification, just considering the supernatant from probiotic fermentation as a postbiotic (Tsilingiri & Rescigno, 2013).

Postbiotic metabolites are gaining interest due to their potential against disease prevention or, even, their treatment. Aguilar-Toalá et al., 2018 highlighted that these metabolic products can modulate blood pressure, inhibit pathogenas colonization, regulate wound healing process, fight against neoplasm development, or increase the antioxidant capacity. Moreover, Foo, Loh, Abdul Mutalib, & Abdul Rahim, 2019 reported the importance of antimicrobial postbiotics and suggested that they could be an interesting substitute for antibiotics. In this sense, this study proved that formulated postbiotic cocktails from *Lactobacillus plantarum* reduced the growth of *Aeromonas hydrophila*, *Enterobacteriaceae*, and other pathogens (Foo, Loh, Abdul Mutalib, & Abdul Rahim, 2019).

Furthermore, postbiotics could be used as an alternative to probiotic supplementation. Despite probiotics are classified as GRAS microorganisms, the ingestion of alive bacteria could trigger undesirable side effects (translocation to other tissues, bacteremia, sepsis, inflammatory response, or resistance genes development) in certain individuals such as young children, immunosuppressed patients, premature neonates, or the elderly (Rad, Maleki, Kafil, & Abbasi, 2021; Wegh, Geerlings, Roeselers, & Belzer, 2019). Tsilingiri et al., 2012 presented the effectiveness of the supernatants obtained from the fermentation of three *Lactobacillus* strains, which were considered the postbiotics, in patients with inflammatory bowel disease. The results showed that the supplementation of probiotics enhanced inflammatory response, however, postbiotics downregulated the inflammatory reaction and conferred protection against *Salmonella* (Tsilingiri et al., 2012).

It is noteworthy that postbiotics could lead to economic savings for food industries compared to probiotics. Probiotics require an important investment to maintain these microorganisms viable and stable to perform the gut colonization after ingestion (Wegh, Geerlings, Roeselers, & Belzer, 2019). On the contrary, postbiotics present a long shelf life up to 5 years, which simplifies the preservation treatments and helps guarantee the quality and food safety (Aguilar-Toalá et al., 2018).

2.1 Postbiotic classification

Postbiotics can be classified according to their molecular nature. The variety of postbiotics can be seen in Fig. 17.1. Amino acids, proteins, vitamins, neurotransmitters, or SCFAs are some of the most relevant postbiotic compounds (Singh, Vishakha, & Singhal, 2018).

2.1.1 Short-chain fatty acids (SCFAs)

Generally, SCFAs are considered to be those carboxylic acids composed by aliphatic tails with a chain length of no more than six carbons. Microorganisms can synthetize these compounds by the anaerobic fermentation of different types of dietary fibers (DF) (Gabriel et al., 2019). Parada Venegas et al., 2019 explained that DF are polymeric carbohydrates nondigestible by the human small intestine. Inulin, resistant starch, pectin, or some types of brans, like oat and wheat, are considered important sources of DF (Parada Venegas et al., 2019). Furthermore, Neis, Cornelis, and Rensen (2015) added that amino acids like glycine (Gly), glutamate, (Glu), threonine (Thr), or aspartate (Asp) could be used as SCFA precursors. For instance, Thr is considered the most significant amino acid for SCFA production due to its ability to be used as a precursor of the three most common SCFAs (Neis et al., 2015).

Among the SCFA group produced by probiotics, acetate, propionate, and butyrate are known as the most influential in human health (Gabriel et al., 2019). *Lactobacillus* species, such as *Lactobacillus buchneri*, *Lactobacillus diolivorans*, or *Lactobacillus reuteri*, are important producers of propionate (Amin, Hashem, Ashour, & Hatti-Kaul, 2013; Zhang, Markus,

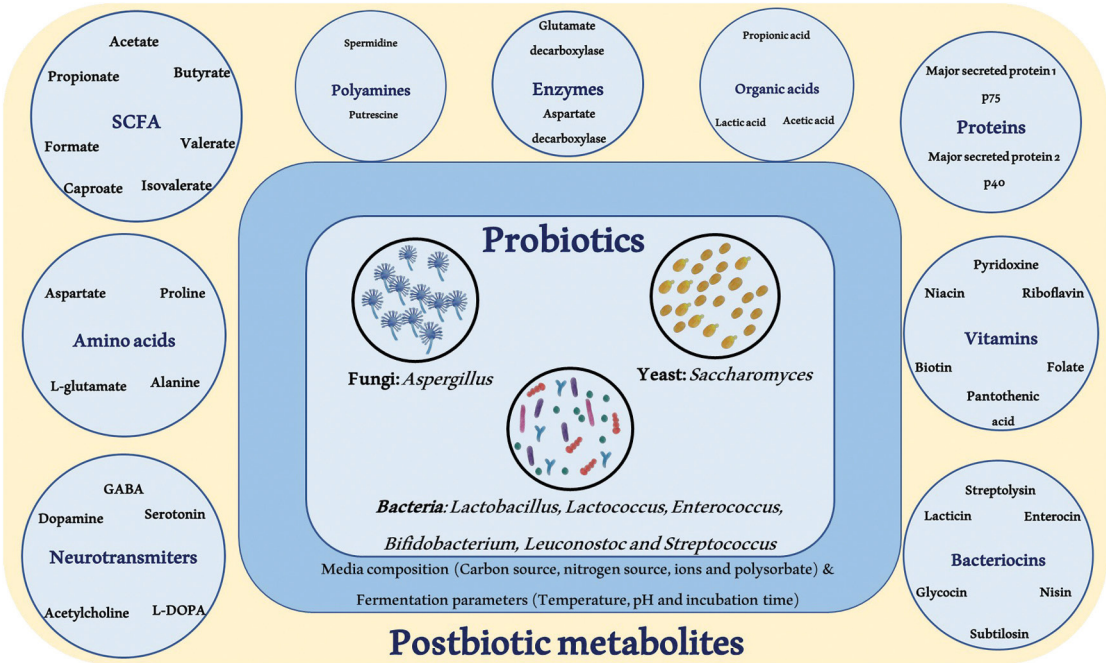


FIGURE 17.1 Summary of the postbiotic metabolites produced by probiotic microorganisms (Singh et al., 2018).

Clarissa, & Michael, 2010), whereas, acetate and butyrate are commonly produced by *Bifidobacterium* species (Parada Venegas et al., 2019).

The biosynthetic process of SCFA begins with the hydrolysis of DF into oligosaccharides and, subsequently, monosaccharides that are converted into the phosphoenolpyruvate (PEP). PEP is common for acetate, butyrate, and propionate biosynthesis. Acetate can be synthesized through the conversion of PEP into pyruvate, which is transformed into acetyl coenzyme A (Acetyl-CoA) and, consequently, Acetyl-CoA is converted into acetate (Ríos-Covián et al., 2016). Likewise, the acetate biosynthesis can be done by using the Wood–Ljungdahl pathway based on the reduction of carbon dioxide into carbon monoxide, which transformation produces Acetyl-CoA and ends in the synthesis of acetate (Parada Venegas et al., 2019).

Besides, propionate can be synthesized by bioconversion of PEP via succinate pathway. However, PEP could be also converted to pyruvate through the acrylate pathway. In this situation, pyruvate is transformed into lactate and its reduction results as propionate (Richards, Li, van Esch, & Garssen, 2016).

Likewise, the biosynthesis of butyrate could be performed via butyrate kinase pathway that is the same as the acetate route till the Acetyl-CoA step (den Besten et al., 2013).

After the production of these three SCFAs, they behave as intestinal protectors with the decreasing of the luminal pH to avoid pathogens colonization (Ríos-Covián et al., 2016). More specifically, butyrate stimulates mucin secretion to block pathogens adhesion. Usually, propionate and acetate are focus on the liver, where they are degraded and used by the hepatocytes (Tan et al., 2014).

2.1.2 Amino acids and proteins

Amino acids can be considered as primary and secondary metabolites because they are intertwined and there is feedback between them (Thirumurugan, Alagappan Cholarajan, Suresh Raja, & Ramasamy, 2018). Among the amino acids classified as essential, probiotics have the ability to synthesize some of them in response to several adverse conditions. Papadimitriou et al., 2016 explained that probiotic like *L. plantarum* or *Lactococcus lactis* can produce Glu, Asp, proline (Pro), and alanine (Ala) in response to osmotic stress. Likewise, amino acids can be biosynthesized against acid stress. Wu, Juan, Guocheng, and Jian (2013) highlighted that *Lactobacillus casei* increases the intracellular accumulation of Asp under low pH, and this amino acid enhances the biomass production of this probiotic.

Some probiotics can go one step further and transformed amino acids into others, which could have different target compared to the precursor amino acids. For instance, after the production of Asp, some *Lactobacilluss,* such as *L. plantarum*, *L. buchneri*, or *Lactobacillus acidophilus*, can increase their resistance against acid environments following the Asp

decarboxylase pathway (AspD). AspD route relieves the acidic stress with the decarboxylation of Asp, as a result, another amino acid is obtained, Ala (Papadimitriou et al., 2016). Other decarboxylase pathways are Glu decarboxylase (GAD) route where Glu is converted into gamma-aminobutyric acid (GABA) or arginine decarboxylation into agmatine (Senouci-Rezkallah, Philippe, & Michel, 2011).

The production of these amino acids as postbiotic compounds could have several beneficial effects in humans because they serve as basic compounds to the synthesis of hormones, neurotransmitters, nucleic acids, or melanin (Aliu, Kanungo, & Arnold, 2018).

Additionally, amino acids can be used by probiotic, such as *L. reuteri* or *L. acidophilus*, to serve as precursors of other beneficial postbiotics. One example is the transformation of tryptophan (Trp) into different indolic acid derivates like indole-3-aldehyde, indole lactic acid, or indole acetic acid (Liu, Alookaran, & Rhoads, 2018). Romani et al., 2014 highlighted the importance of indoles and their antiinflammatory effect, mainly against fungal and yeast infections.

Some research has reported that probiotic bacteria like *Lactobacillus* spp. can even produce full proteins classified as postbiotics. Cicenia et al., 2014 explained that *Lactobacillus rhamnosus* can synthesize two proteins known as p40 and p75 as their molecular mass is around 40 and 75 kDa, respectively. These proteins are considered the first soluble proteins obtained from probiotics. Studies have shown that both proteins can modulate the homeostasis of the intestinal epithelium, inhibit apoptosis, and prevent damage generation in cells and tissues by the tumor necrosis factor (Cicenia et al., 2014).

2.1.3 Neurotransmitters

Neurotransmitters are considered essential chemical compounds in humans since they regulate the neural signaling to ensure the proper body–brain homeostasis (Mora, Segovia, de Blas, & Del Arco, 2012). Surprisingly, some of the most important neurotransmitters can be synthesized by probiotics but they use these compounds in a different way such as the protection against stressful situations, that is, at low pH. Among the neurotransmitters synthesized by probiotics, GABA, serotonin, and dopamine can be highlighted (Ali & Haq, 2010; Wu, Tun, Law, Khafipour, & Shah, 2017).

GABA is a nonprotein amino acid classified as an inhibitory neurotransmitter that mainly works in the central nervous system (CNS) (Sarasa et al., 2019). In most of the situations, *Lactobacillus* spp. uses the GAD pathway to produce GABA. Briefly, this biosynthetic pathway is focused on the decarboxylation of the precursor Glu or its salt monosodium glutamate (MSG). Some probiotics, such as *Aspergillus oryzae*, can use a completely different route where putrescine is used as precursor (Diez-Gutiérrez et al., 2020).

In the same way, other neurotransmitters like serotonin can be produced by probiotics like *L. lactis*, *L. plantarum*, or *Streptococcus thermophilus* (Liang et al., 2019) using Trp as precursor molecule. This amino acid is modified to a 5-hydroxy-tryptophan (5-HTP) by the action of a Trp hydrolase and serotonin is then obtained by bioconversion of 5-HTP catalyzed by an aromatic amino acid decarboxylase (O'Mahony, Clarke, Borre, Dinan, & Cryan, 2015).

Moreover, *A. oryzae* is able to synthesize 3,4-dihydroxyphenyl Ala, better known as L-DOPA, which has positive effects against Parkinson's disease. The enzymatic oxidation of tyrosine (Try) enhances the production of L-DOPA (Ali & Haq, 2010) and some probiotics like *L. lactis* can even use L-DOPA as a precursor of another essential neurotransmitter, dopamine (Vodolazov, Dbar, Oleskin, & Stoyanova, 2018).

2.1.4 Vitamins

Vitamins are micronutrients that play a key role in human health but the inability to produce these compounds forces humans to obtain them exogenously. Several LAB and bifidobacteria can biosynthesize B-group vitamins (Thakur, Sudhir, & Sachinandan, 2016).

Riboflavin, also known as vitamin B_2, presents high relevance in the preservation and restoration of important body structures such as mucous membranes, connecting tissues, neural, or immune system (Ibrahim, Hoda, Kawther, & Sharaf, 2015). Generally, the microbial production of this vitamin involves seven consecutive steps where guanosine triphosphate (GTP) is used as the precursor compound. The process starts with the hydrolysis of the imidazole ring of the GTP and its bioconversion to 5-amino-6-ribitylamino2,4-pyrimidinedione (ARP). Afterward, ARP is transformed by consecutive deamination, reduction of the side chain, phosphorylation, and condensation, which as a result 6,7-dimethyl-8-ribityllumazine (DR). Finally, riboflavin is obtained after dismutation of DR molecule (Bacher, Eberhardt, Fischer, Kis, & Richter, 2000). Thakur et al. (2016) summarized the probiotic bacteria that can release riboflavin naturally. *L. acidophilus*, *Bacillus subtilis*, *Lactobacillus fermentum*, and *L. plantarum* can biosynthesize this vitamin in several dairy products. Riboflavin, also known as vitamin B_2, presents high relevance in the preservation and restoration of important body structures such as mucous membranes, connecting tissues, neural or immune system (Ibrahim et al., 2015). Generally, the microbial production of this vitamin involves seven consecutive steps where GTP is used as the precursor compound. The process starts with the

hydrolysis of the imidazole ring of the GTP and its bioconversion to ARP. Afterward, ARP by deamination, reduction of the side chain, phosphorylation, and condensation consecutive reactions is converted into DR. Finally, riboflavin is obtained after dismutation of DR molecule (Bacher, Eberhardt, Fischer, Kis, & Richter, 2000). Thakur et al. (2016) summarized the probiotic bacteria that can release riboflavin naturally. For instance, *L. acidophilus*, *B. subtilis*, *L. fermentum*, and *L. plantarum* can biosynthesize this vitamin in several dairy products (Thakur et al., 2016).

Folate, vitamin B_{11}, is another remarkable vitamin obtained from probiotic secondary metabolism. This vitamin is interesting due to its participation in the reparation, methylation, and replication of DNA, as well as its implication in illnesses of different etiology such as Alzheimer's, cancer, or coronary diseases, caused by decrease in folate concentration (Ibrahim et al., 2015). The folate biosynthetic pathway is a complex mixture of enzymatic reactions influenced mainly by the stepwise grouping of pteridine, para-aminobenzoic acid (pABA), and Glu. Briefly, the GTP transformation through different steps produces 6-hydroxymethyl-7,8-dihydropterin pyrophosphate (DHPPP) that is linked to pABA by the action of dihydropteroate (DHP) synthase. The fusion of both DHPPP and pABA forms a DHP molecule that is subsequently glutamylated, reduced, and ends up in folate (Rad, Khosroushahi, Khalili, & Jafarzadeh, 2016). Leblanc et al., 2011 reported that *S. thermophilus*, *Bifidobacterium animalis*, or *L. lactis* are able to synthesize high folate concentration.

LAB can produce in less quantity other vitamins from the B-group. Al-Fataftah Abdur Rahman, Herzallah, Mabood, and Alshawabkeh (2013) studied the ability of different LAB to produce vitamin B_{12} (cobalamin) and B_6 (pyridoxine). The results showed that *L. reuteri* obtained from the rumen of goat and camel presented a significant production of these two vitamins (Al-Fataftah et al., 2013). Likewise, Hamzehlou, Abbas, Sedigheh, and Hosseini (2018) evaluated the B-vitamin production of several *Lactobacillus* strains isolated from yogurt reporting that vitamin B_6 and B_9 can be largely synthesized by *Lactobacillus paracasei*. However, vitamin B_3 (niacin) is mainly produced by *L. acidophilus*, and vitamin B_2 by *L. fermentum* (Hamzehlou et al., 2018).

3 Conditions of probiotics to produce postbiotics

Postbiotic production from probiotics depends on precursor concentration, media composition, temperature, pH, or the probiotic incubation time. The effect of each parameter is linked to each probiotic strain and, therefore, these conditions should be specifically adjusted to get the postbiotic target (Diez-Gutiérrez et al., 2020).

3.1 Culture media composition

In general, precursor molecules present a direct relationship between its concentration and the production of postbiotic. For example, Mahara, Lilis, and Hanifah (2019) reported that folate production by most LAB cultures was related to the supplementation of pABA because LAB cannot synthesize de novo this molecule. As well, Shan et al., 2015 indicated that the supplementation of MSG is essential for the synthesis of GABA.

However, some precursor molecules can enhance the synthesis of postbiotics by indirect overstimulation of other biosynthetic routes (Demain, 1998).

Therefore, culture media composition should be balanced between precursor molecule and other additives such as carbon and nitrogen source, cofactors, or polysorbates (Chen et al., 2015a).

Glucose is considered an excellent carbon source because it is easy to metabolize and increases the biomass. In the synthesis of postbiotics, the glucose concentration must be properly set because a high concentration could lead to consuming all this sugar in the growth phase and, in consequence, inhibiting secondary metabolism pathways. Hence, an optimized glucose concentration is required to slow down microorganism growth and focusing on postbiotic production (Ruiz, Chávez, Forero, & García-Huante, 2010). Chen, Wenwen, and Xinmo (2015b) assessed the effect of different carbon sources like glucose, lactose, sucrose, and soluble starch, for GABA synthesis by *L. plantarum*. The results showed higher GABA concentration when glucose was used (Chen et al., 2015a). Zareian, Ebrahimpour, Sabo Mohamed, & Saari, 2013 also considered glucose as the best carbon source for GABA production and they found that the highest amount of GABA was obtained with 6% of glucose.

Nevertheless, glucose is not always the best choice for postbiotic production. For instance, Hernandez-Hernandez et al., 2012 reported that lactulose and galacto-oligosaccharides (GOS) obtained from lactose or lactulose were the best carbon sources for the synthesis of SFCA by some *Lactobacillus* strains.

Moreover, the carbon source supplied can be combined with different nitrogen sources. Wang et al., 2018 evaluated the effects of 36 different nitrogen sources in the biosynthesis of GABA by *Lactobacillus brevis*. The results showed that most nitrogen compounds did not affect GABA production and only some, such as the yeast extracts, increased GABA production (Wang et al., 2018). Ooi et al., 2015 investigated how different nitrogen sources can affect the production of

antimicrobial postbiotics by *L. plantarum.* Interestingly, the combination of glucose and yeast extract increased the concentration of postbiotics, whereas neither glucose and meat extract nor glucose and a cocktail of yeast, peptone and meat extract, had any significant effect on the postbiotic production (Ooi et al., 2015). Ali & Haq, 2010 also studied the effect of different nitrogen sources in the production of L-DOPA by *Aspergillus niger* showing that 6% of glucose combined with 1.5% of peptone and 1% of yeast extract achieved the highest yield.

Another type of additives in the culture medium can have an impact on postbiotic biosynthesis. An example is the emulsifier Tween 80 that increases the membrane fluidization with the incorporation of oleic acid to it and, in consequence, enhances the absorption of nutrients (Foo, Loh, Abdul Mutalib, & Abdul Rahim, 2019). In this sense, Saraniya et al. (2014) showed that Tween 80 is an essential compound for bacteriocin production. Malheiros, Voltaire, Svetoslav, and Bernadette (2015) also highlighted the importance of this emulsifier for bacteriocin production by *Enterococcus faecium* and also reported that other polysorbate emulsifiers such as Tween 20 can enhance the biosynthesis of bacteriocins by *Lactobacillus sakei*.

Regarding other culture medium components, ions and micronutrients added in low amount could improve the postbiotic synthesis. For example, vitamin B_{12} biosynthesis is specifically influenced by cobalt and several minor nutrients such as tripotassium phosphate, manganese chloride, or sodium phosphate (Kośmider, Bialas, Kubiak, Drozdzynska, & Czaczyk, 2012). Lim et al., 2018 studied the effect of different chemical reagents and coenzymes on the expression of GAD enzyme used in GABA synthesis. Among coenzymes, pyridoxal-5-phosphate (PLP), pyridoxal hydrochloride, and pyridoxine were added to determine which of these compounds have the highest impact on GABA yield. The highest amount of GABA was obtained using PLP followed by pyridoxine and pyridoxal hydrochloride. Focusing on chemical reagents, better results were obtained using calcium chloride, ammonium sulfate, or manganese chloride, among others (Lim et al., 2018).

3.2 Cultivation parameters

Temperature, incubation time, and pH of the culture medium can play a key role in the postbiotic synthesis and, therefore, the optimization of these parameters in combination with other conditions can enhance the postbiotic production (Zhang, Zeng, Tan, Tang, & Xiang, 2017). Leblanc et al., 2011 reported high concentration of folate obtained at 30°C during 4 days of incubation. Additionally, the intracellular pH could affect folate production depending on the probiotic strain. For example, acidic environments could help *S. thermophilus* to produce more amount of folate but this condition was not relevant for *L. lactis* (Leblanc et al., 2011). Min, Kyungmoon, Don, and Young Je (2015) found that the synthesis of L-DOPA could be maximized at pH 8 and 40°C allowing *Bacillus* sp. *JPJ* to achieve a 99.4% bioconversion of Tyr into L-DOPA.

Miao et al., 2015 performed an optimization of the production of an antimicrobial postbiotic by *L. paracasei.* The best results were shown after 24 h of incubation, at 30°C and an initial pH of 7 (Miao et al., 2015). Zareian et al., 2012 reported high concentration of Glu after 96 h of incubation, at 30°C, and 4.5 as initial pH. Likewise, Tajabadi et al., 2015 determined that *L. plantarum* produced more GABA after 60 h of incubation, at 36°C, and an initial pH around 5.

4 Human health benefits of probiotics and postbiotics

Microbiota is the term used to designate microorganisms that live in a specific environment, called itself a microbiome. These microorganisms can be commensal, symbiotic, and pathogenic bacteria, fungi, and viruses. In the case of microorganisms that grow in the intestine, they are called the gut microbiota. Recent studies have investigated that the microbiome is capable of modulating behavior by connecting the neuroendocrine and immune systems (Sylvia et al., 2018). In addition, the existence of the so-called gut–brain axis has been demonstrated, in which the microbiota of the digestive tract and the CNS are bidirectionally connected. In this regard, this connection is believed to occur through three pathways: the vagus nerve, the systemic pathway (by releasing hormones, metabolites, and neurotransmitters), and the immune system (by the action of cytokines), as it can see in Fig. 17.2 (Molska & Reguła, 2019; Gómez-Eguílaz, José, Laura, & Blanco, 2019).

In a healthy gut microbiota, there are about 500 different species, bacteria, and fungi that cause disease along with beneficial bacteria. If the latter bacteria prevail, the intestinal physiology is normal, and the overall body status is healthy. Among the causes that may cause the disappearance of beneficial bacteria from the intestinal microbiota are stress, infections, antibiotic treatments, inadequate diets, etc.

Dysbiosis is the imbalance between gut microorganisms that generates a deteriorated microbiota. Pathogenic bacteria, viruses, yeasts, and fungi grow out of control, often leading to a rise in allergies and autoimmune conditions (Fig. 17.2) (Lyte, 2014).

Previous studies reported that intestinal microbiota is responsible for producing neurotransmitters such as dopamine, serotonin, and norepinephrine (Lyte, 2011), and that these compounds can directly reach the brain through the vagus nerve

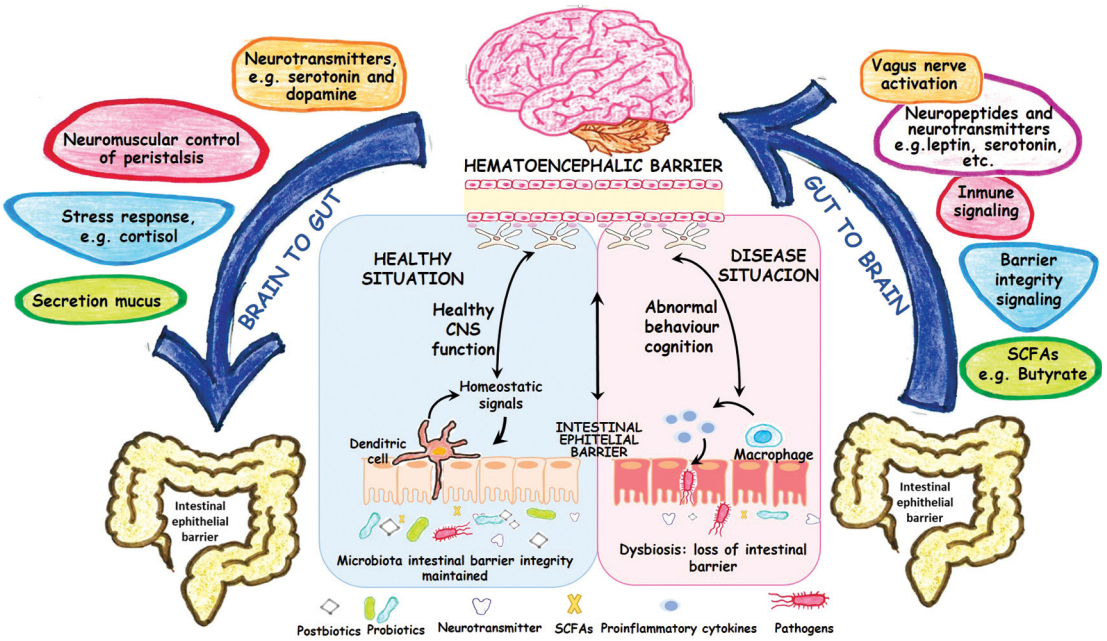


FIGURE 17.2 Gut–brain connection. Scheme depicting the gut–brain axis connections that can be activated or modulated by stress and diseases.

(Perez-Burgos et al., 2013; Borovikova et al., 2000). Also, high levels of neurotransmitter precursors such as Trp have been linked to gut microbiota (Desbonnet, Garrett, Clarke, Bienenstock, & Dinan, 2008), as well as the activation of neuroreceptors associated with appetite control, pain sensation, mood, and memory (Muccioli et al., 2010). In 2017 Anderson, Cryan, & Dinan, 2017 created the term "psychobiotics" to explain the benefit of consuming adequate amount of live probiotics for a good psychological health. Since then, this term has been expanded to include both probiotic microorganisms and postbiotic compounds.

Currently, there are a large number of scientific studies that demonstrate the relationship between probiotics and postbiotics and their effect on human physiology and health, as it can see in Table 17.3.

Although many unknown aspects still need to be clarified, the gut–brain axis is postulated as responsible for numerous neurological disorders of great health impact, such as Alzheimer's disease, Parkinson's disease, or multiple sclerosis, as well as other diseases such as cancer, diabetes, asthma, and intestinal bowel disease.

Currently, scientific studies, such as cell cultures, in animal models, human clinical trials, are underway trying to assess the impact of probiotics and postbiotics on some of these diseases.

5 Application of probiotics and postbiotics for healthy food development

Once the beneficial effects of the consumption of probiotics and postbiotics have been recognized, it is necessary to define the way in which they will be consumed. In the market, there are different examples of the commercial strategies that are chosen both in the pharmaceutical industry (in the parapharmacy product line) and in the food industry: nutraceutical formulations in the form of capsules, tablets, dispersible powders, etc. or as ingredients that are incorporated in food and beverages.

The introduction of probiotics in food formulation has found no barriers for consumers, since food fermentation is an ancient strategy to extend the shelf life of food and also involves a flavor and texture modification of the raw material creating and interesting food and cultural heritage: cheese (historically from Middle East), miso (Japan), wine (Zagros mountains), vinegar (Roman empire), chili sauce (Mexico), kombucha tea (China), sauerkraut (Germany), kimchi (Korea), fermented fish (Asia), garri (Nigeria), etc.

In addition, nowadays, fermented foods are related to healthy diets and modern cuisine. According to Kerry Health and Nutrition Institute (2020), the fermented food product market is projected to reach $689.34 billion by 2023, and the CAGR (Compound Annual Growth Rate) is expected to be between 4.3 and 7%, being Europe and United States the largest markets and Asia-pacific the fastest growing one. In fact, there is a positive perspective on fermented foods and beverages from a large part of the consumers with food taste and health aspects important purchase incentives.

TABLE 17.3 Probiotics and postbiotics with beneficial human health effects.

Probiotic/Postbiotic		Disease	Human health effect	References
PROBIOTICS				
Probiotics	*Lactobacillus acidophilus*	Intestinal bowel disease, colorectal cancer Crohn's disease, ulcerative colitis L.	Enhances immune response ↑*Lactobacilli*, *Bifidobacteria* ↓ *Staphylococcus aureus*	Chen et al. (2015b), Chen, Zou, & Lian, (2013), Khazaie et al., (2012), Park et al., (2018)
	Lactobacillus casei BL23	Colorectal cancer	Expand gut Treg cells Enhances immune response	Jacouton, Chain, Sokol, Langella, & Bermúdez-Humarán, (2017), Lozano-Ojalvo, Leblanc, & Bermúdez-Humarán, (2016)
	Lactobacillus fermentum FTDC 812	Hypercholesterolemia	↑*Lactobacillus*	Lye et al., (2017)
	Lactobacillus johnsonii	Acute live injury	↑IL-22, *Lactobacillus*	Nakamoto et al., (2017)
	Lactobacillus plantarum CCFM10, RS15-3	Oxidative stress	↑*Bacteroidetes*, *Firmicutes*	Zhao et al., (2018)
	L. acidophilus Lactobacillus rhamnosus, *Bifidobacterium bifidum*	Type 2 diabetes	↑*Firmicutes*, *Actinobacteria* ↓*Bacteroidetes*	Bagarolli et al., (2017)
	Bifidobacterium breve IPLA20004 E.	Inflammatory	Enhances immune response	Sánchez et al., (2015)
POSTBIOTICS				
SCFA	Short-chain fatty acids	Multiple sclerosis	Promote T-cell differentiation toward regulatory subtypes (Treg cells)	Zeng, Gong, Liu, & Chen, (2019)
	Short-chain fatty acids	Type 2 diabetes	Enhance glucose homeostasis and insulin effectiveness	Mandaliya et al. (2019)
	Butyrate	Autoimmune diseases	Facilitating neuronal plasticity and long-term memory formation Restoring cognitive function	Haghikia et al., (2015)
	Propionate	Inflammatory diseases	Protective effects against lipopolysaccharides (LPS), induced blood–brain barrier disruption, and oxidative stress	Chen et al., (2017), Hoyles et al., (2018)
Protein/peptides/amino acids	Tryptophan	Intestinal bowel disease	5-Hydroxytryptamine, kynurenine, and aryl hydrocarbon receptor pathways	Agus, Planchais, & Sokol, (2018)

(*Continued*)

TABLE 17.3 Probiotics and postbiotics with beneficial human health effects. (*Cont.*)

Probiotic/Postbiotic		Disease	Human health effect	References
Neurotransmitters	GABA	Heart attack and stroke	Hypotensive effect	Abd El-Fattah, Sakr, El-Dieb, & Elkashef, (2018), Cáceres, (2017)
	GABA	Huntington's disease Alzheimer's disease	Inhibits neurotransmission	Fuhrer et al., (2017), Hsu, Chang, & Chern, (2018), Mele, Rui, and Duarte (2019)
	GABA	Anxiety and depression	Relaxant and antidepressant effect	Boonstra et al., (2015), Bravo et al., (2011), Soussan et al. (2016)
	GABA	Epilepsy	Reduce seizure severity	Bagheri, Heydari, Alinaghipour, & Salami, (2019)
	GABA	Diabetes type 1	α-Cells: GABA induces membrane hyperpolarization and inhibits glucagon secretion. β-Cells: GABA induces membrane depolarization and enhances insulin secretion	Tian, Lu, Zhang, & Chau, (2014), Qinghua, Gerald, and Yun (2015)
	GABA	Cancer	Delays and/or inhibits cancer cell proliferation Stimulatory action on cancer cell apoptosis Potent tumor suppressor	Brzozowska, (2017), Song et al., (2016), Wang et al. (2016b)
	GABA	Asthma	Control in asthma Enhances immunity	Yocum et al., (2017), Forkuo et al., (2017)
	L-DOPA	Parkinson's disease	Control dopamine deficiency	Min et al. (2015)
	Dopamine	Neural diseases	Regulation neurochemical pathways	Lyte (2018)
	Acetylcholine	Alzheimer's disease	Control acetylcholine deficiency and cholinergic receptors alteration	Nimgampalle et al. (2017)
	Acetylcholine and serotonin	Cancer	Regulation neural signaling and inflammatory response	Gayathri (2016)

One of the health benefits perceived by consumers is the transformation of raw food components to make healthier foods by postbiotic compound production. The ingestion of FODMAP (Fermentable Oligo-, Di-, Monosaccharides And Polyols) is related to gastrointestinal disturbances and a low FODMAP diet could be especially targeted at people with functional bowel disorders such as irritable bowel syndrome (Ramírez, Tejero Mas, Gato Núñez, Rivera Jiménez, & Román Vargas, 2018). FODMAP includes components present in different foods and beverages, such as milk or plant-based ones: fructans and GOS, lactose, fructose, and polyalcohols. Food fermentation is an alternative for removing FODMAP in foods (Nyyssölä, Simo, Emilia, & Poutanen, 2020). Thus *Saccharomyces cerevisiae* activity contributes to reduce the FODMAP content in dough during fermentation. Fermentation is also a useful strategy to reduce the presence of GOS in legumes, such as soybean fermented products (tofu, tempeh, miso, soy sauce, etc.) or the lactose reduction in fermented dairy products such as yogurt, fermented milk, or cheese by LAB activity.

On the other hand, consumers also appreciate fermented foods as healthy due to the ability of microorganisms to synthesize bioactive compounds. For example, *Acetobacter* and *Gluconobacter* oxidize ethanol from alcoholic beverages to produce vinegar and the acetic acid produced has been related to the glucose metabolism, showing a beneficial effect on the glycemic profile (Santos, Moraes, da Silva, & Prestes, 2019).

The healthier perception of fermented foods is driving an important evolution in the food and beverage marketplace, particularly for dairy products. However, plant products are increasing rapidly in the global food market and according to Allied Market Research (2019), vegan food market size is expected to reach $31.4 billion by 2026. Nowadays, the food and beverage industry is working to standardize traditional and new fermented products in order to produce healthy fermented foods according to consumer demands under safety and quality requirements in their target markets (Adewumi, 2019; IsamMohamed Ahmed & FahadAl-Juhaimi, 2019). But also, scientific and technological advances are being to improve fermented food products to the market.

However, to ensure the functionality of new products, it is necessary to ensure the concentration of probiotics and postbiotics in the selected dosage form. This implies that the active compounds must not interact with other components of the product matrix throughout its useful life and it must remain unchanged after ingestion.

One remarkable technology applied to improve probiotics and postbiotics functionality is the encapsulation. Microencapsulation of functional components is a process of entrapping components within one or more classes of shell materials to fabricate a capsule, typically a few microns in diameter referred to microcapsules. The microencapsulation is used to enhance nutritional value, mask off-flavor, facilitate storage, and extend shelf life without adverse influence on the food physical, chemical, or functional properties (Ye, Nicolas, & Selomulya, 2018). Nanoencapsulation is generally defined as the design, production, and application of structures, devices, and systems, through control of the shape and size of the material between 1 and 100 nm in order to achieve the delivery of poorly bioactive compounds into functional food ingredients (Bazana, Codevilla, & de Menezes, 2019).

Currently, there is an extensive number of encapsulation technologies that can serve to encapsulate probiotics to optimize their viability both in the fermentation process and in the final product for controlled release in the intestine (Chávarri, Izaskun, & Maria, 2012). Maintenance and viability of starter culture in fermented foods is still an immense challenge for the food industry. Starter culture encapsulation provides protection to the cells increasing the viability of the delivered amount (Kavitake, Sujatha, Palanisamy, & Shetty, 2018) or the delivery of metabolites produces by the encapsulated cell (Barbosa, Todorov, Jurkiewicz, & Bernadette, 2015; Lindner et al., 1998), but also opens possibilities to optimize selected probiotic starters for other applications (Plessas et al., 2007).

Furthermore, these micro- and nanoencapsulation technologies can be used also to improve the stability of postbiotic compounds and their bioavailability. Microencapsulated postbiotics could avoid degradative reactions during storage, such as oxidation (Rasti, Arezoo, & Selamat, 2017); on the other hand, the application of enteric coating materials provides gastric resistance to minimize the degradation of postbiotic compounds in the gastric acid medium (Puccetti, Giovagnoli, Zelante, Romani, & Ricci, 2018); and encapsulation is also used to control the delivery of postbiotic compounds in a specific targeted point of the human body (Fontes et al., 2018). All encapsulating materials must be food grade or pharmaceutical grade to develop a food ingredient or nutritional complement dosage form, and they are normally selected between polysaccharides, proteins, lipids, waxes, etc. These materials are appropriately selected regarding the encapsulation technology and the desired release mechanism (Table 17.4).

Moreover, encapsulation technologies are a useful tool to adjust the dosage of functional ingredients when a probiotic culture must be dosed with a specified amount of prebiotic ingredient (Marañon García, San Vicente Laurent, Hidalgo Lemus, & Chavarri, 2016) or metabolite (Eratte et al., 2015), as well as to accurately dose a defined proportion of several cultures (Divya et al., 2015).

As mentioned previously, beyond their relevance in the scientific field, postbiotics and probiotics are important for food industry. According to the report published by Grand View Research in April 2019, the global functional food market size was

TABLE 17.4 Examples of some encapsulation strategies for postbiotics.

Postbiotic	Material	Encapsulation technology	References
SCFA	Cellulose nanocrystals, sunflower oil	Pickering emulsions	Du Le, Simon, Loveday, and Sarkar (2020)
	Lecithin, sunflower oil	Nanoliposomes	Ghorbanzade, Seid Mahdi, Sahar, and Hadavi (2017)
	PEG, cholesterol, phosphatidylcholine	Liposomes	Rasti et al. (2017)
	Whey protein isolate, Arabic gum	Complex coacervation	Eratte et al., (2015)
Amino acids; peptides	Chitosan	Gelation	Danish, Vozza, Byrne, Frias, & Ryan, (2017)
	Niosomes; Liposomes	Nanovesicles	Rezvani et al., (2019)
	Maltodextrin	Spray-drying	Akbarbaglu et al., (2019)
	Whey protein	Gelation	O'Neill, Egan, Jacquier, O´Sullivan, & O´Riordan, (2014)
Neurotransmitters	PEG, cholesterol, phosphatidylcholine	Liposomes	Fontes et al., (2018)
	Chitosan	Ionic gelation method	Shilpa, Mary, Malat, and Paulose (2013)
Vitamins	Whey protein, starch	Gelation	Liu et al., (2020)
	Phospholipids	Nanoliposomes	Hamadou, Wen-Can, Changhu, and Xiangzhao (2020)
	Ovalbumin, pectin	Complex coacervation	Xiang et al., (2020)
	Triglycerides, lecithin, surfactants, etc.	Nanoemulsion	Maurya and Aggarwal (2019)

estimated at $161.49 billion in 2018, and it is projected to reach $275.77 billion in 2025 (FFMW, 2019). Regarding functional ingredients, the probiotic market size was estimated at $2.09 billion in 2018 with a projected CAGR of 7.9% for the period 2019–25 (Grand View Research, 2020). Postbiotics are not recognized as functional ingredients but they are relevant considering that, for example, SCFAs or vitamins are some of the most relevant ingredients to determine food market perspectives.

6 Conclusions

Probiotics are currently in the spotlight of the food field due to the wide variety of potential beneficial effects particularly useful in maintaining human welfare. Likewise, the research and production of postbiotics are evolving in such a way that these beneficial metabolites are gaining importance because their broad spectrum of health action combined with the effectiveness of probiotics goes one step further in the development of new functional foods.

Acknowledgment

This work was supported by the Basque government (grant ELKARTEK—KK-2019/00034).

References

Abd El-Fattah, A., Sakr, S, El-Dieb, S, & Elkashef, H (2018). Developing functional yogurt rich in bioactive peptides and gamma-aminobutyric acid related to cardiovascular health. *LWT*, *98*(February), 390–397. doi: 10.1016/j.lwt.2018.09.022.

Adewumi, G. A. (2019). Health-promoting fermented foods. *Encyclopedia of Food Chemistry*, 399–418. doi: 10.1016/B978-0-08-100596-5.21774-5.

Aguilar-Toalá, J. E., Garcia-Varela, R., Garcia, H. S., Mata-Haro, V., González-Córdova, A. F., Vallejo-Cordoba, B., et al. (2018). Postbiotics: An evolving term within the functional foods field. *Trends in Food Science & Technology*, *75*, 105–114. doi: 10.1016/J.TIFS.2018.03.009.

Agus, A., Planchais, J, & Sokol, H (2018). Gut microbiota regulation of tryptophan metabolism in health and disease. *Cell Host and Microbe*, *23*(6), 716–724. doi: 10.1016/j.chom.2018.05.003.

Akbarbaglu, Z., Mahdi Jafarib, S, Sarabandi, K, Mohammadi, M, Khakbaz Heshmatia, M, Pezeshki, A, et al. (2019). Influence of spray drying encapsulation on the retention of antioxidant properties and microstructure of flaxseed protein hydrolysates. *Colloids and Surfaces B: Biointerfaces*, *178*, 421–429. doi: 10.1016/J.COLSURFB.2019.03.038.

Al-Fataftah, A. R. A., Herzallah, S. M., Mabood, F., & Alshawabkeh, K. (2013). Enrichment of vitamin B12 and B6 and lowering cholesterol levels of eggs by lactic acid bacteria. *Journal of Food, Agriculture and Environment*, *11*(2), 674–678.

Ali, S., & Haq, I. (2010). Production of 3,4-dihydroxy L-phenylalanine by a newly isolated *Aspergillus niger* and parameter significance analysis by Plackett-Burman design. *BMC Biotechnology*, *10*(86), 1–8. doi: 10.1186/1472-6750-10-86.

Aliu, E., Kanungo, S, & Arnold, G. L. (2018). Amino acid disorders. *Annals of Translational Medicine*, *6*(24), 471. doi: 10.21037/atm.2018.12.12.

Amara, A. A., & Shibl, A. (2015). Role of probiotics in health improvement, infection control and disease treatment and management. *Saudi Pharmaceutical Journal*, *23*(2), 107–114. doi: 10.1016/J.JSPS.2013.07.001.

Amin, H. M., Hashem, A. M., Ashour, M. S., & Hatti-Kaul, R. (2013). 1,2 Propanediol utilization by *Lactobacillus reuteri* DSM 20016, role in bioconversion of glycerol to 1,3 propanediol, 3-hydroxypropionaldehyde and 3-hydroxypropionic acid. *Journal of Genetic Engineering and Biotechnology*, *11*(1), 53–59. doi: 10.1016/j.jgeb.2012.12.002.

Anderson, S. C., Cryan, J. F., & Dinan, T. (2017). *The psychobiotic revolution: Mood, food, and the new science of the gut-brain connection* (pp. 1–355). National Geographic. https://books.google.com/books?hl=es&lr=&id=dLe-DQAAQBAJ&oi=fnd&pg=PA9&ots=C2P-d91FvE&sig=T2Tqo2wjsKwUg8DdkrEhHuz7qrw (April 14, 2020).

Angmo, K., Kumari, A., Savitri, & Bhalla, T. C. (2016). Probiotic characterization of lactic acid bacteria isolated from fermented foods and beverage of Ladakh. *LWT—Food Science and Technology*, *66*, 428–435. doi: 10.1016/j.lwt.2015.10.057.

Armas, F., Camperio, C., & Marianelli, C. (2017). In vitro assessment of the probiotic potential of *Lactococcus lactis* LMG 7930 against ruminant mastitis-causing pathogens. *PLoS One*, *12*(1), e0169543. doi: 10.1371/journal.pone.0169543.

Azad, M., Kalam, A., Sarker, M., Li, T., & Yin, J. (2018). Probiotic species in the modulation of gut microbiota: An overview. *BioMed Research International*, *2018*, 9478630. doi: 10.1155/2018/9478630.

Bacher, A., Eberhardt, S, Fischer, M, Kis, K, Richter, G, et al. (2000). Biosynthesis of vitamin B2 (RIBOFLAVIN). *Annual Review of Plant Physiology and Plant Molecular Biology*, *20*(1), 153–167. doi: 10.1016/j.abb.2008.02.008.

Bagarolli, R. A., Tobar, N, Oliveira, A. G., Araújo, T. G., Carvalho, B. M., Rocha, G. Z., et al. (2017). Probiotics modulate gut microbiota and improve insulin sensitivity in DIO mice. *Journal of Nutritional Biochemistry*, *50*, 16–25. doi: 10.1016/j.jnutbio.2017.08.006.

Bagheri, S., Heydari, A., Alinaghipour, A., & Salami, M. (2019). Effect of probiotic supplementation on seizure activity and cognitive performance in PTZ-induced chemical kindling. *Epilepsy and Behavior*, *95*, 43–50. doi: 10.1016/j.yebeh.2019.03.038.

Barbosa, M. S., Todorov, S. D., Jurkiewicz, C. H., & Bernadette, D. G. M. F. (2015). Bacteriocin production by *Lactobacillus curvatus* MBSa2 entrapped in calcium alginate during ripening of salami for control of listeria monocytogenes. *Food Control*, *47*, 147–153. doi: 10.1016/j.foodcont.2014.07.005.

Bazana, M. T., Codevilla, C. F., & de Menezes, C. R. (2019). Nanoencapsulation of bioactive compounds: Challenges and perspectives. *Current Opinion in Food Science*, *26*, 47–56. doi: 10.1016/j.cofs.2019.03.005.

Behbahani, B. A., Noshad, M., & Falah, F. (2019). Inhibition of *Escherichia Coli* adhesion to human intestinal Caco-2 cells by probiotic candidate *Lactobacillus plantarum* strain L15. *Microbial Pathogenesis*, *136*, 103677. doi: 10.1016/j.micpath.2019.103677.

Boonstra, E., de Kleijn, R., Colzato, L. S., Alkemade, A., Forstmann, B. U., Nieuwenhuis, S., et al. (2015). Neurotransmitters as food supplements: The effects of GABA on brain and behavior. *Frontiers in Psychology*, *6*(OCT), 6–11. doi: 10.3389/fpsyg.2015.01520.

Borovikova, L. V., Ivanova, S., Zhang, M., Yang, H., Botchkina, I., Watkins, L. R., et al. (2000). Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature*, *405*(6785), 458–462. doi: 10.1038/35013070.

Bravo, J. A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T. G., et al. (2011). Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(38), 16050–16055. doi: 10.1073/pnas.1102999108. http://www.ncbi.nlm.nih.gov/pubmed/21876150.

Brzozowska, A., et al. (2017). γ-Amino butyric acid (GABA) level as an overall survival risk factor in breast cancer. *Annals of Agricultural and Environmental Medicine*, *24*(3), 435–439. doi: 10.26444/aaem/75891.

Cáceres, P. J., et al. (2017). Enhancement of biologically active compounds in germinated brown rice and the effect of sun-drying. *Journal of Cereal Science*, *73*, 1–9. doi: 10.1016/j.jcs.2016.11.001.

Chávarri, M., Marañon, I., Ares, R., Ibañez, F. C., Marzo, F., Villaran, M. C., et al. (2010). Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions. *International Journal of Food Microbiology*, *142*(1–2), 185–189. http://www.ncbi.nlm.nih.gov/pubmed/20659775.

Chávarri, M., Marañón, I., & Villarán, M. C. (2012). Encapsulation technology to protect probiotic bacteria. *Probiotics*. InTech.

Chen, L. L., Zou, Y. Y., Lian, G. H., et al. (2013). Efficacy profiles for different concentrations of *Lactobacillus acidophilus* in experimental colitis. *World Journal of Gastroenterology*, *19*(32), 5347–5356.

Chen, L., et al. (2015a). *Lactobacillus acidophilus* suppresses colitis-associated activation of the IL-23/Th17 axis. *Journal of Immunology Research*, *2015*, 909514. http://www.ncbi.nlm.nih.gov/pubmed/25973440 (April 14, 2020).

Chen, W., Xu, W., & Zheng, X. (2015b). A *Lactobacillus plantarum* strain newly isolated from Chinese sauerkraut with high γ-aminobutyric acid productivity and its culture conditions optimization. *Metallurgical and Mining Industry*, *7*(9), 388–393.

Chen, X., Su, W., Wan, T., Yu, J., Zhu, W., Tang, F., et al. (2017). Sodium butyrate regulates Th17/Treg cell balance to ameliorate uveitis via the Nrf2/HO-1 pathway. *Biochemical Pharmacology*, *142*, 111–119. http://www.ncbi.nlm.nih.gov/pubmed/28684304 (April 14, 2020).

Chubukov, V., Gerosa, L., Kochanowski, K., & Uwe, S. (2014). Coordination of microbial metabolism. *Nature Reviews Microbiology*, *12*, 327–340. http://dx.doi.org/10.1038/nrmicro3238.

Cicenia, A., Scirocco, A., Carabottu, M., Pallotta, L., Marignani, M., Severi, C., et al. (2014). Postbiotic activities of lactobacilli-derived factors. *Journal of Clinical Gastroenterology*, *48*(December), S18–S22.

Craney, A., Ahmed, S., & Nodwell, J. (2013). Towards a new science of secondary metabolism. *The Journal of Antibiotics*, *66*, 387–400.

Danish, M. K., Vozza, G., Byrne, H. J., Frias, J. M., Ryan, S. M., et al. (2017). Comparative study of the structural and physicochemical properties of two food derived antihypertensive tri-peptides, Isoleucine-Proline-Proline and Leucine-Lysine-Proline encapsulated into a chitosan based nanoparticle

system. *Innovative Food Science & Emerging Technologies*, *44*, 139–148. https://www.sciencedirect.com/science/article/pii/S146685641630577X?viewFullText=true (April 28, 2020).

den Besten, G., van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D-J, Bakker, B. M., et al. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research*, *54*(9), 2325–2340.

Demain, A. L. (1998). Induction of microbial secondary metabolism. *International Microbiology*, *1*(4), 259–264.

Desbonnet, L., Garrett, L., Clarke, G., Bienenstock, J., Dinan, T. G., et al. (2008). The probiotic bifidobacteria infantis: An assessment of potential antidepressant properties in the rat. *Journal of Psychiatric Research*, *43*(2), 164–174.

Diez-Gutiérrez, L., San Vicente, L., Barrón, L. J. R., del Carmen Villarán, M., & Chávarri, M. (2020). Gamma-aminobutyric acid and probiotics: Multiple health benefits and their future in the global functional food and nutraceuticals market. *Journal of Functional Foods*, *64*, 1–14.

Divya, J. B., & Nampoothiri, K. M. (2015). Encapsulated *Lactococcus lactis* with enhanced gastrointestinal survival for the development of folate enriched functional foods. *Bioresource Technology*, *188*, 226–230. https://www.sciencedirect.com/science/article/pii/S0960852415000930?viewFullText=true (April 28, 2020).

Du Le, H., Loveday, S. M., Singh, H., & Sarkar, A. (2020). Gastrointestinal digestion of Pickering emulsions stabilised by hydrophobically modified cellulose nanocrystals: Release of short-chain fatty acids. *Food Chemistry*, *320*, 126650. https://www.sciencedirect.com/science/article/pii/S0308814620305124?viewFullText=true (April 28, 2020).

Eratte, D., McKnight, S., Gengenbach, T. R., Dowling, K., Barrow, C. J., Adhikari, B. P., et al. (2015). Co-encapsulation and characterisation of omega-3 fatty acids and probiotic bacteria in whey protein isolate–gum Arabic complex coacervates. *Journal of Functional Foods*, *19*, 882–892. https://www.sciencedirect.com/science/article/pii/S1756464615000419 (April 28, 2020).

FAO/WHO. (2006). *Probiotics in food: Health and nutritional properties and guidelines for evaluation: Report of a Joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria, and Joint FAO/WHO Working Group on drafting guidelines for the evaluation of probiotics in food*. (85, pp. 1–50). Rome, Italy: Food and Agriculture Organization of the United Nations, World Health Organization. 0254-4725.

FDA. (2018). *Microorganisms & microbial-derived ingredients used in food* (p. WEB). U.S. Food and Drug. https://www.fda.gov/food/generally-recognized-safe-gras/microorganisms-microbial-derived-ingredients-used-food-partial-list.

Fontes, Marco, Vaz, G. C., Cardoso, T. Z. D., De Oliveira, M. F., Campagnole-Santos, M. J., & Souza dos Santos, R. A., et al. (2018). GABA-containing liposomes: Neuroscience applications and translational perspectives for targeting neurological diseases. *Nanomedicine: Nanotechnology, Biology and Medicine*, *14*(3), 781–788. https://www.sciencedirect.com/science/article/pii/S1549963417305853?viewFullText=true (April 16, 2020).

Foo, H. L., Loh, T. C., Abdul Mutalib, N. E, Abdul Rahim, R., et al. (2019). The myth and therapeutic potentials of postbiotics. *Microbiome and metabolome in diagnosis, therapy, and other strategic applications* (pp. 210–211). Amsterdam, the Netherlands: Elsevier Inc. http://dx.doi.org/10.1016/B978-0-12-815249-2.00021-X.

Forkuo, G. S., Nieman, A. N., Yuan, N. Y., Kodali, R., Yu, O. B., Zahn, N. M., et al. (2017). Alleviation of multiple asthmatic pathologic features with orally available and subtype selective GABAA receptor modulators. *Molecular Pharmaceutics*, *14*(6), 2088–2098. https://doi.org/10.1021/acs.molpharmaceut.7b00183.

Fuhrer, T. E., Palpagama, T. H., Waldvogel, H. J., Synek, B. J. L., Turner, C., Faull, R. L., et al. (2017). Impaired expression of GABA transporters in the human Alzheimer's disease hippocampus, subiculum, entorhinal cortex and superior temporal gyrus. *Neuroscience*, *351*, 108–118. http://www.sciencedirect.com/science/article/pii/S0306452217302075.

FFMW. (2019). *Functional foods market worth $275.7 billion by 2025 | CAGR: 7.9%*. Grand View Research. https://www.grandviewresearch.com/press-release/global-functional-foods-market (May 6, 2020).

Gabriel, F. C., & Fantuzzi, G. (2019). The association of short-chain fatty acids and leptin metabolism: A systematic review. *Nutrition Research*, *72*, 18–35. https://doi.org/10.1016/j.nutres.2019.08.006.

Gayathri, D. (2016). *Anti-cancer properties of probiotics: A natural strategy for cancer prevention* (226th ed., 190, p.1). Semantic scholar, Corpus ID: 212570975. https://www.semanticscholar.org/paper/Anti-Cancer-Properties-of-Probiotics-%3A-A-Natural-Gayathri/9fafbc6fa17c99fe3d53132ca4131e061a0caad2 (January 18, 2019).

Georgieva, M., Peikova, L., Andonova, L., Zlatkov, A., et al. (2014). PROBIOTICS – HEALTH BENEFITS, CLASSIFICATION, QUALITY ASSURANCE AND QUALITY CONTROL – REVIEW. *PHARMACIA*, *61*(4), 22–31. http://bsphs.org/wp-content/uploads/2015/01/Georgieva.pdf (May 5, 2020).

Ghorbanzade, T., Jafari, S. M., Akhavan, S., & Hadavi, R. (2017). Nano-encapsulation of fish oil in nano-liposomes and its application in fortification of yogurt. *Food Chemistry*, *216*, 146–152. https://www.sciencedirect.com/science/article/pii/S0308814616312523?viewFullText=true (April 28, 2020).

Gómez-Eguílaz, M., Ramón-Trapero, J. L., Pérez-Martínez, L., & Blanco, J. R. (2019). The microbiota-gut-brain axis and its great projections. *Revista de Neurologia*, *68*(3), 111–117. https://www.neurologia.com/articulo/2018223 (April 13, 2020).

Gomi, A., Yamaji, K., Yoshioka, M., Miyazaki, K., Iwama, Y., Urita, Y., et al. (2018). *Bifidobacterium bifidum* YIT 10347 fermented milk exerts beneficial effects on gastrointestinal discomfort and symptoms in healthy adults: A double-blind, randomized, placebo-controlled study. *Journal of Dairy Science*, *101*(6), 4830–4841. http://www.sciencedirect.com/science/article/pii/S0022030218302625.

Grand View Research. (2020). *Probiotic ingredients market size & share, global industry report, 2025*. www.grandviewresearch.com: Grand View Research. https://www.grandviewresearch.com/industry-analysis/probiotic-ingredients-market (May 6, 2020).

Haghikia, A., Jörg, S., Duscha, A., Berg, J., Manzel, A., Waschbisch, A, et al. (2015). Dietary fatty acids directly impact central nervous system autoimmunity via the small intestine. *Immunity*, *43*(4), 817–829.

Hamzehlou, P., Sepahy, A. A., Mehrabian, S., & Hosseini, F. (2018). Production of vitamins B3, B6 and B9 by *Lactobacillus* isolated from traditional yogurt samples from 3 cities in Iran, winter 2016. *Applied Food Biotechnology*, *5*(2), 105–118.

Hamadou, A. H., Huang, W. -C., Xue, C., & Mao, X. (2020). Formulation of vitamin C encapsulation in marine phospholipids nanoliposomes: Characterization and stability evaluation during long term storage. *LWT*, *127*, 109439. https://www.sciencedirect.com/science/article/pii/S002364382030428X (April 28, 2020).

Hernandez-Hernandez, O., Muthaiyan, A., Moreno, F. J., Montilla, A., Sanz, M. L., Ricke, S. C., et al. (2012). Effect of prebiotic carbohydrates on the growth and tolerance of *Lactobacillus*. *Food Microbiology*, *30*(2), 355–361. http://dx.doi.org/10.1016/j.fm.2011.12.022.

Hoyles, L., Snelling, T., Umlai, U. K., Nicholson, J. K., Carding, S. R., GLEN, R. C., et al. (2018). Microbiome–Host systems interactions: Protective effects of propionate upon the blood–brain barrier. *Microbiome*, *6*(55), 1–13.

Hsu, Y. T., Chang, Y. G., & Chern, Y. (2018). Insights into $GABA_A$ ergic system alteration in Huntington's disease. *Open Biology*, *8*(12), 1–19. doi: 10.1098/rsob.180165.

Ibrahim, G. A., El-Sayed, H. S., El-Shafei, K., & Sharaf, O. M. (2015). Riboflavin and folate production in different media using encapsulated *Streptococcus thermophilus* and *Lactobacillus plantarum*. *Middle East Journal of Applied Sciences*, *5*(2013), 663–669.

Jacouton, E., Chain, F., Sokol, H., Langella, P., Bermúdez-Humarán, L. G., et al. (2017). Probiotic strain *Lactobacillus casei* BL23 prevents colitis-associated colorectal cancer. *Frontiers in Immunology*, *8*(NOV), 1–10. doi: 10.3389/fimmu.2017.01553.

Kavitake, D., Kandasamy, S., Bruntha Devi, P., & Shetty, P. H. (2018). Recent developments on encapsulation of lactic acid bacteria as potential starter culture in fermented foods – A review. *Food Bioscience*, *21*, 34–44. https://www.sciencedirect.com/science/article/pii/S2212429217302961?viewFullText=true (April 16, 2020).

Kechagia, M., Basoulis, D., Konstantopoulou, S., Dimitriadi, D., Gyftopoulou, K., Skarmoutsou, N., et al. (2013). Health benefits of probiotics: A review. *ISRN Nutrition*, *2013*, 481651. http://www.ncbi.nlm.nih.gov/pubmed/24959545 (April 26, 2019).

Kerry Health and Nutrition Institute. (2020). Fermented foods: Stacking up the science. *Food ingredients 1st*. Foodingredientsfirts.com. https://www.foodingredientsfirst.com/Webinars/fermented-foods-stacking-up-the-science.html (May 6, 2020).

Khazaie, K., Zadeh, M., Khan, M. W., Bere, P., Gounari, F., Dennis, K, et al. (2012). Abating colon cancer polyposis by *Lactobacillus acidophilus* deficient in lipoteichoic acid. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(26), 10462–10467.

Suchita, K. (2017). Isolation of lactic acid bacteria and detection of their antimicrobial. *Internal Journal on Emerging Technologies*, *8*(1), 260–266.

Papadimitriou, K., Alegría, A., Bron, P. A., de Angelis, M., Gobbetti, M., & Kleerebezem, M., et al. (2016). Stress physiology of lactic acid bacteria. *Microbiology and Molecular Biology Reviews*, *80*(3), 837–890.

Kośmider, A., Bialas, W., Kubiak, P., Drozdzynska, A., Czaczyk, K., et al. (2012). Vitamin B 12 production from crude glycerol by *Propionibacterium freudenreichii* ssp. shermanii: Optimization of medium composition through statistical experimental designs. *Bioresource Technology*, *105*, 128–133.

Leblanc, J. G., Laiño, J. E., Juarez de l Valle, M., Vannini, V., van Sinderen, D., Taranto, M. P., et al. (2011). B-Group vitamin production by lactic acid bacteria—Current knowledge and potential applications. *Journal of Applied Microbiology*, *111*(6), 1297–1309.

Lozano-Ojalvo, D., Leblanc, J. G., & Bermúdez-Humarán, L. G., et al. (2016). *Lactobacillus casei* BL23 regulates Treg and Th17 T-cell populations and reduces DMH-associated colorectal cancer. *Journal of Gastroenterology*, *51*(9), 862–873. http://www.ncbi.nlm.nih.gov/pubmed/26749362 (April 14, 2020).

Liang, H., Dai, Z., Kou, J., Sun, K., Chen, J., Yang, Y., et al. (2019). Dietary L-tryptophan supplementation enhances the intestinal mucosal barrier function in weaned piglets: Implication of tryptophan-metabolizing microbiota. *International Journal of Molecular Sciences*, *20*(1), 1–13.

Lim, H. S., Seo, D. H., Cha, I. T., Lee, H., Nam, Y. D., Seo, N. M., et al. (2018). Expression and characterization of glutamate decarboxylase from *Lactobacillus brevis* HYE1 isolated from kimchi. *World Journal of Microbiology and Biotechnology*, *34*(3), 1–10. http://dx.doi.org/10.1007/s11274-018-2427-6.

Lin, T. -L., Shu, C. C., Lai, W. F., Tzeng, C. M., Lai, H. C., Lu, C. C., et al. (2019). Investiture of next generation probiotics on amelioration of diseases – Strains do matter. *Medicine in Microecology*, *1–2*, 100002.

Lin, X., Xia, Y., Yang, Y., Wang, G., Zhou, W., Ai, L., et al. (2020). Probiotic characteristics of *Lactobacillus plantarum* AR113 and Its molecular mechanism of antioxidant. *LWT*, *126*, 109278. doi: 10.1016/j.lwt.2020.109278. http://www.sciencedirect.com/science/article/pii/S0023643820302668.

Lindner, M. D., & Emerich, D. F. (1998). Therapeutic potential of a polymer-encapsulated l-DOPA and dopamine-producing cell line in rodent and primate models of Parkinson's disease. *Cell Transplantation*, *7*(2), 165–174. https://www.sciencedirect.com/science/article/abs/pii/S0963689797001693 (April 28, 2020).

Liu, K., Kong, X. L., Li, Q. M., Zhang, H. L., Zha, X. Q., & Luo, J. P., et al. (2020). Stability and bioavailability of vitamin D3 encapsulated in composite gels of whey protein isolate and lotus root amylopectin. *Carbohydrate Polymers*, *227*, 115337. https://www.sciencedirect.com/science/article/pii/S0144861719310045?viewFullText=true (April 28, 2020).

Liu, Y., Alookaran, J. J., & Rhoads, J. M. (2018). Probiotics in autoimmune and inflammatory disorders. *Nutrients*, *10*(10), 1537. doi: 10.3390/nu10101537.

Lye, H. S., Kato, T., Low, W. Y., Taylor, T. D., Prakash, T., Lew, L. C., et al. (2017). *Lactobacillus fermentum* FTDC 8312 combats hypercholesterolemia via alteration of gut microbiota. *Journal of Biotechnology*, *262*, 75–83.

Lyte, M. (2011). Probiotics function mechanistically as delivery vehicles for neuroactive compounds: Microbial endocrinology in the design and use of probiotics. *BioEssays*, *33*(8), 574–581. http://doi.wiley.com/10.1002/bies.201100024 (April 14, 2020).

Lyte, M. (2014). Microbial endocrinology and the microbiota-gut-brain axis. *Advances in Experimental Medicine and Biology*, *817*, 3–24.

Lyte, M. (2018). *Dopamine production in Enterococcus faecium: A microbial endocrinology-based mechanism for the selection of probiotics based on neurochemical-producing potential*, *28*, 1–10. doi: 10.1371/journal.pone.0207038.

Madigan, M., Bender, K. S., Buckley, D. H., Sattley, B. W., et al. (2019). *Brock biology of microorganisms* (15th ed., pp. 1–350). GLOBAL EDITION: Pearson.

Mahara, F. A., Nuraida, L., & Nuryani Lioe, H. (2019). Fermentation of milk using folate-producing lactic acid bacteria to increase natural folate content: A review. *Journal of Applied Biotechnology Reports*, *6*(4), 129–136.

Malheiros, P. S., Sant'Anna, V., Todorov, S. D., & Franco, B. D. G. M. (2015). Optimization of growth and bacteriocin production by *Lactobacillus sakei* subsp. Sakei2a. *Brazilian Journal of Microbiology*, *46*(3), 825–834.

Mandaliya, D. K., & Seshadri, S. (2019). Short chain fatty acids, pancreatic dysfunction and type 2 diabetes. *Pancreatology*, *19*(2), 280–284. https://doi.org/10.1016/j.pan.2019.01.021.

Marañon García, I., San Vicente Laurent, L., Hidalgo Lemus, N., & Chavarri, M. (2016). *Multilayer probiotic microcapsules* (EP16382056). EUROPEAN: TECNALIA RESEARCH & INNOVATION.

Marinelli, F., & Marcone, G. L. (2011). Small molecules: Microbial secondary metabolites *Comprehensive biotechnology* (Vol. 3). (2nd ed., pp.1–15). Amsterdam, the Netherlands: Elsevier B.V. http://dx.doi.org/10.1016/B978-0-08-088504-9.00539-0.

Maurya, V. K., & Aggarwal, M. (2019). A phase inversion based nanoemulsion fabrication process to encapsulate vitamin D3 for food applications. *The Journal of Steroid Biochemistry and Molecular Biology*, *190*, 88–98. https://www.sciencedirect.com/science/article/pii/S0960076019300305?viewFullText=true (April 28, 2020).

Mele, M., Costa, R. O., & Duarte, C. B. (2019). Alterations in GABAA-receptor trafficking and synaptic dysfunction in brain disorders. *Frontiers in Cellular Neuroscience*, *13*(March), 1–16.

Miao, J., Xu, M., Guo, H., He, L., Gao, X., DiMarco-Crook, C., et al. (2015). Optimization of culture conditions for the production of antimicrobial substances by probiotic *Lactobacillus paracasei* subsp. tolerans FX-6. *Journal of Functional Foods*, *18*, 244–253. http://dx.doi.org/10.1016/j.jff.2015.07.011.

Min, K., Park, K., Park, D. H., & Je Yoo, Y. (2015). Overview on the biotechnological production of L-DOPA. *Applied Microbiology and Biotechnology*, *99*(2), 575–584.

Isam A. Mohamed Ahmed, Fahad Y. Al-Juhaimi, Alaa El-Din Ahmed Bekhit, Fermentation of Grains, Encyclopedia of Food Chemistry, 10.1016/B978-0-08-100596-5.21657-0, (107-116), (2019). Crossref

Molska, M., & Reguła, J. (2019). Potential mechanisms of probiotics action in the prevention and treatment of colorectal cancer. *Nutrients*, *11*(10), 2453. doi: 10.3390/nu11102453.

Mora, F., Segovia, G., de Blas, M., Del Arco, A., et al. (2012). Stress, neurotransmitters, corticosterone and body-brain integration. *Brain Research*, *1476*, 71–85. http://dx.doi.org/10.1016/j.brainres.2011.12.049.

Muccioli, G. G., Naslain, D., Bäckhed, F., Reigstad, C. S., Lambert, D. M., Delzenne, N. M., et al. (2010). The endocannabinoid system links gut microbiota to adipogenesis. *Molecular Systems Biology*, *6*(1), 392. https://onlinelibrary.wiley.com/doi/abs/10.1038/msb.2010.46 (April 14, 2020).

Nakamoto, N., Amiya, T., Aoki, R., Saito, H., Hattori, M., Kanai, T., et al. (2017). Commensal *Lactobacillus* controls immune tolerance during acute liver injury in mice. *Cell Reports*, *21*(5), 1215–1226.

Neis, E. P. J. G., Dejong, C. H. C., & Rensen, S. S. (2015). The role of microbial amino acid metabolism in host metabolism. *Nutrients*, *7*(4), 2930–2946.

Nimgampalle, M., & Kuna, Y. (2017). Anti-alzheimer properties of probiotic, *Lactobacillus plantarum* MTCC 1325 in Alzheimer's disease induced albino rats. *Journal of Clinical and Diagnostic Research*, *11*(8), KC01–KC05.

Nyyssölä, A., Ellilä, S., Nordlund, E., & Poutanen, K. (2020). Reduction of FODMAP content by bioprocessing. *Trends in Food Science & Technology*, *99*, 257–272. https://www.sciencedirect.com/science/article/pii/S0924224419309252 (April 13, 2020).

O'Mahony, S. M., Clarke, G., Borre, Y. E., Dinan, T. G., & Cryan, J. F. (2015). Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behavioural Brain Research*, *277*, 32–48. http://dx.doi.org/10.1016/j.bbr.2014.07.027.

O'Neill, G. J., Egan, T., Jacquier, J. C., O´Sullivan, M., & O´Riordan, E. D. (2014). Whey microbeads as a matrix for the encapsulation and immobilisation of riboflavin and peptides. *Food Chemistry*, *160*, 46–52. https://www.sciencedirect.com/science/article/pii/S0308814614003872?viewFullText=true (April 28, 2020).

Ooi, M. F., Mazlan, N., Foo, H. L., Loh, T. C., Mohamad, R., Rahim, R. A., et al. (2015). Effects of carbon and nitrogen sources on bacteriocin-inhibitory activity of postbiotic metabolites produced by *Lactobacillus plantarum* I-UL4. *Malaysian Journal of Microbiology*, *11*(2), 176–184. (January 2017).

Park, J. S., Choi, J. W., Jhun, J. Y., Kwon, J. Y., Lee, B. I., Yang, C. W., et al. (2018). *Lactobacillus acidophilus* improves intestinal inflammation in an acute colitis mouse model by regulation of Th17 and treg cell balance and fibrosis development. *Journal of Medicinal Food*, *21*(3), 215–224.

Park, S. Y., & Lim, S. D. (2015). Probiotic characteristics of *Lactobacillus plantarum* FH185 isolated from human feces. *Korean Journal for Food Science of Animal Resources*, *35*(5), 615–621. http://www.ncbi.nlm.nih.gov/pubmed/26761889 (April 5, 2020).

Perez-Burgos, A., Wang, B., Mao, Y. K., Mistry, B., Neufeld, K. A. M., Bienenstock, J., et al. (2013). Psychoactive bacteria *Lactobacillus rhamnosus* (JB-1) elicits rapid frequency facilitation in vagal afferents. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *304*(2), G211–G220. https://www.physiology.org/doi/10.1152/ajpgi.00128.2012 (April 14, 2020).

Plessas, S., Trantallidi, M., Bekatorou, A., Kanellaki, M., Nigam, P., Koutinas, A. A., et al. (2007). Immobilization of kefir and *Lactobacillus casei* on brewery spent grains for use in sourdough wheat bread making. *Food Chemistry*, *105*(1), 187–194. http://www.sciencedirect.com/science/article/pii/S0308814607003111 (March 31, 2014).

Puccetti, M., Giovagnoli, S., Zelante, T., Romani, L., Ricci, M., et al. (2018). Development of novel indole-3-aldehyde–loaded gastro-resistant spray-dried microparticles for postbiotic small intestine local delivery. *Journal of Pharmaceutical Sciences*, *107*(9), 2341–2353. https://www.sciencedirect.com/science/article/abs/pii/S0022354918302624 (April 16, 2020).

Rad, A. H., Khosroushahi, A. Y., Khalili, M., & Jafarzadeh, S. (2016). Folate bio-fortification of yoghurt and fermented milk: A review. *Dairy Science and Technology*, *96*(4), 427–441. http://dx.doi.org/10.1007/s13594-016-0286-1.

Rad, A. H., Maleki, L. A., Kafil, H. S., & Abbasi, A. (2021). Postbiotics: A novel strategy in food allergy treatment. *Critical Reviews in Food Science and Nutrition*, *61*(3), 492–499. doi: 10.1080/10408398.2020.1738333.

Ramírez, F. B., Tejero Mas, M., Gato Núñez, C., Rivera Jiménez, N., & Román Vargas, M., et al. (2018). La Alimentación En El Síndromedel Intestino Irritabletie. *FMC—Formación Médica Continuada en Atención Primaria*, *25*(7), 422–432. https://www.sciencedirect.com/science/article/abs/pii/S1134207218301245?via%3Dihub (April 13, 2020).

Rasti, B., Erfanian, A., & Selamat, J. (2017). Novel nanoliposomal encapsulated omega-3 fatty acids and their applications in food. *Food Chemistry*, *230*, 690–696. https://www.sciencedirect.com/science/article/pii/S0308814617304715?viewFullText=true (April 16, 2020).

Rezvani, M., Hesari, J., Peighambardoust, S. H., Manconi, M., Hamishehkar, H., Escribano-Ferrer, E., et al. (2019). Potential application of nanovesicles (niosomes and liposomes) for fortification of functional beverages with isoleucine-proline-proline: A comparative study with central composite design approach. *Food Chemistry*, *293*, 368–377. https://www.sciencedirect.com/science/article/pii/S0308814619308106?viewFullText=true (April 28, 2020).

Richards, L. B., Li, M., van Esch, B. C. A. M., & Garssen, J. (2016). The effects of short-chain fatty acids on the cardiovascular system. *PharmaNutrition*, *4*(2), 68–111.

Ríos-Covián, D., Ruas-Madiedo, P., Margolles, A., Gueimonde, M., de los Reyes-Gavilán, C. G., & Salazar, N. (2016). Intestinal short chain fatty acids and their link with diet and human health. *Frontiers in Microbiology*, *7*(FEB), 1–9.

Romani, L., Zelante, T., De Luca, A., Iannitti, R. G., Moretti, S., Bartoli, A., et al. (2014). Microbiota control of a tryptophan-AhR pathway in disease tolerance to fungi. *European Journal of Immunology*, *44*(11), 3192–3200.

Ruiz, B., Chávez, A., Forero, A., & García-Huante, Y. (2010). Production of microbial secondary metabolites: Regulation by the carbon source. *Critical Reviews in Microbiology*, *36*(2), 146–167.

Sánchez, B., Gonzalez-Rodriguez, I., Arboleya, S., López, P., Suárez, A, & Ruas-Madiedo, P., et al. (2015). The effects of *Bifidobacterium breve* on immune mediators and proteome of HT29 cells monolayers. *BioMed research international*, *2015*, 479140. http://www.ncbi.nlm.nih.gov/pubmed/25793196 (April 14, 2020).

Santos, H. O., Moraes, W. M., da Silva, G. A. R., & Prestes, J. (2019). Vinegar (acetic acid) intake on glucose metabolism: A narrative review. *Clinical Nutrition ESPEN*, *32*, 1–7. https://www.sciencedirect.com/science/article/pii/S2405457719303055 (April 13, 2020).

Saraniya, A., & Jeevaratnam, K. (2014). Optimization of nutritional and non-nutritional factors involved for production of antimicrobial compounds from *Lactobacillus pentosus* SJ65 using response surface methodology. *Brazilian Journal of Microbiology*, *45*(1), 81–88.

Sarasa, Sabna B., Mahendra, R., Muthusamy, G., Thankappan, B., Raja Femil Selta, D., & Angayarkanni, J., et al. (2019). A brief review on the non-protein amino acid, gamma-amino butyric acid (GABA): Its production and role in microbes. *Current Microbiology*, *77*(4), 534–544. doi: 10.1007/s00284-019-01839-w.

Senouci-Rezkallah, K., Schmitt, P., & Jobin, M. P. (2011). Amino acids improve acid tolerance and internal PH maintenance in bacillus cereus ATCC14579 strain. *Food Microbiology*, *28*(3), 364–372.

Shan, Y., Man, C. X., Han, X., Li, L., Guo, Y., Deng, Y., et al. (2015). Evaluation of improved γ-aminobutyric acid production in yogurt using *Lactobacillus plantarum* NDC75017. *Journal of Dairy Science*, *98*(4), 2138–2149.

Shilpa, J., Abraham Pretty, M, Anitha, M., & Paulose, C. S. (2013). Gamma aminobutyric acid B and 5-hydroxy tryptamine 2A receptors functional regulation during enhanced liver cell proliferation by GABA and 5-HT chitosan nanoparticles treatment. *European Journal of Pharmacology*, *715*(1–3), 154–163. https://www.sciencedirect.com/science/article/pii/S0014299913004354?viewFullText=true (April 28, 2020).

Singh, A., Vishwakarma, V., & Singhal, B. (2018). Metabiotics: The functional metabolic signatures of probiotics: Current state-of-art and future research priorities—metabiotics: Probiotics effector molecules. *Advances in Bioscience and Biotechnology*, *09*(04), 147–189. http://www.scirp.org/journal/doi.aspx?DOI=10.4236/abb.2018.94012.

Song, L., Du, A., Xiong, Y., Jiang, J., Zhang, Y., & Tian, Z., et al. (2016). Gamma-aminobutyric acid inhibits the proliferation and increases oxaliplatin sensitivity in human colon cancer cells. *Tumor Biology*, *37*(11), 14885–14894. https://doi.org/10.1007/s13277-016-5367-5.

Soussan, C., & Kjellgren, A. (2016). The users of novel psychoactive substances: Online survey about their characteristics, attitudes and motivations. *International Journal of Drug Policy*, *32*, 77–84. http://dx.doi.org/10.1016/j.drugpo.2016.03.007.

Sylvia, K. E., & Demas, G. E. (2018). A gut feeling: Microbiome-brain-immune interactions modulate social and affective behaviors. *Hormones and Behavior*, *99*, 41–49.

Tajabadi, N., Ebrahimpour, A., Baradarn, A., Rahim, R. A., Mahyudin, N. A., Manap, M. Y. A., et al. (2015). Optimization of γ-aminobutyric acid production by *Lactobacillus plantarum* Taj-Apis362 from honeybees. *Molecules*, *20*(4), 6654–6669.

Tan, J., McKenzie, C., Potamitis, M., Thorburn, A. N., Mackay, C. R., & Macia, L., et al. (2014). The role of short-chain fatty acids in health and disease *Advances in immunology the role of short-chain fatty acids in health and disease* (Vol. 121). (1st ed., pp. 91–119). Amsterdam, the Netherlands: Elsevier Inc. http://dx.doi.org/10.1016/B978-0-12-800100-4.00003-9.

Thakur, K., Tomar, S. K., & De, S. (2016). Lactic acid bacteria as a cell factory for riboflavin production. *Microbial Biotechnology*, *9*(4), 441–451.

Thirumurugan, Durairaj, Alagappan Cholarajan, Suresh Raja, & Ramasamy, Vijayakumar (2018). An Introductory Chapter: Secondary Metabolites." Secondary metabolites-. *Sources and Applications*, 3–21https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics.

Tian, J., Lu, Y., Zhang, H., Chau, C. H., et al. (2014). γ-aminobutyric acid inhibits T cell autoimmunity and the development of inflammatory responses in a mouse type 1 diabetes model. *The Journal of Immunology*, *173*(8), 5298–5304.

Tsilingiri, K., & Rescigno, M. (2013). Postbiotics: What else? *Beneficial Microbes*, *4*(1), 101–107. http://www.ncbi.nlm.nih.gov/pubmed/23271068 (April 8, 2020).

Tsilingiri, K., Barbosa, T., Penna, G., Capriolo, F., Sonzogni, A., & Viale, G., et al. (2012). Probiotic and postbiotic activity in health and disease: Comparison on a novel polarised ex-vivo organ culture model. *Gut*, *61*(7), 1007–1015.

Tulumoğlu, Ş., İbrahim Kaya, H., & Şimşek, Ö. (2014). Probiotic characteristics of *Lactobacillus fermentum* strains isolated from Tulum cheese. *Anaerobe*, *30*, 120–125. https://linkinghub.elsevier.com/retrieve/pii/S1075996414001371 (April 5, 2020).

Parada Venegas, D., De la Fuente, M. K., Landskron, G., Gonzalez, M. J., Quera, R., & Dijkstra, G., et al. (2019). Short chain fatty acids (SCFAs) mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Frontiers in Immunology*, *10*(MAR), 1–16. doi: 10.3389/fimmu.2019.00277.

Vodolazov, I. R., Dbar, S. D., Oleskin, A. V., & Stoyanova, L. G. (2018). Exogenous and endogenous neuroactive biogenic amines: Studies with *Lactococcus lactis* subsp. lactis. *Applied Biochemistry and Microbiology*, *54*(6), 603–610.

Wang, C., et al. (2016a). γ-Aminobutyric acid inhibits the growth of cholangiocarcinoma via CAMP/PKA signal pathway. *International Journal of Clinical and Experimental Medicine*, *9*(6), 9992–9998.

Qinghua, W., Prud'homme, G., & Wan, Y. (2015). GABAergic system in the endocrine pancreas: A new target for diabetes treatment. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, *8*, 79. http://www.dovepress.com/gabaergic-system-in-the-endocrine-pancreas-a-new-target-for-diabetes-t-peer-reviewed-article-DMSO (May 29, 2019).

Wang, Q., Liu, X., Fu, J., Wang, S., Chen, Y., Chang, K., et al. (2018). Substrate sustained release-based high efficacy biosynthesis of GABA by *Lactobacillus brevis* NCL912. *Microbial Cell Factories*, *17*(1), 1–8. https://doi.org/10.1186/s12934-018-0919-6.

Wang, Y., et al. (2016b). Probiotic potential of *Lactobacillus paracasei* FM-LP-4 isolated from Xinjiang camel milk yoghurt. *International Dairy Journal*, *62*(62), 28–34.

Wegh, C. A. M., Geerlings, S. Y., Roeselers, G., Belzer, C., et al. (2019). Postbiotics and their potential applications in early life nutrition and beyond. *International Journal of Molecular Sciences*, *20*(19), 1–23. doi: 10.3390/ijms20194673.

Winkelströter, L. K., Tulini, F. L., & De Martinis, E. C. P. (2015). Identification of the bacteriocin produced by cheese isolate *Lactobacillus paraplantarum* FT259 and its potential influence on listeria monocytogenes biofilm formation. *LWT—Food Science and Technology*, *64*(2), 586–592.

Wu, C., Zhang, J., Du, G., & Chen, J. (2013). Aspartate protects *Lactobacillus casei* against acid stress. *Applied Microbiology and Biotechnology*, *97*(9), 4083–4093.

Wu, Q., Tun, H. M., Law, Y. S., Khafipour, E., & Shah, N. P. (2017). Common distribution of gad operon in *Lactobacillus brevis* and its GadA contributes to efficient GABA synthesis toward cytosolic near-neutral PH. *Frontiers in Microbiology*, *8*(FEB), 1–16.

Xiang, C., Gao, J., Ye, H., Ren, G., Ma, X., Xie, H., et al. (2020). Development of ovalbumin-pectin nanocomplexes for vitamin D3 encapsulation: Enhanced storage stability and sustained release in simulated gastrointestinal digestion. *Food Hydrocolloids*, *106*, 105926. https://www.sciencedirect.com/science/article/pii/S0268005X19330140?viewFullText=true (April 28, 2020).

Ye, Q., Georges, N., & Selomulya, C. (2018). Microencapsulation of active ingredients in functional foods: From research stage to commercial food products. *Trends in Food Science & Technology*, *78*, 167–179. https://www.sciencedirect.com/science/article/pii/S092422441830013X?viewFullText=true (April 28, 2020).

Yocum, G. T., Turner, D. L., Danielsson, J., Barajas, M. B., Zhang, Y., Xu, D., et al. (2017). GABA a receptor α 4-subunit knockout enhances lung inflammation and airway reactivity in a murine asthma model. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *313*(2), L406–L415.

Zareian, M., Ebrahimpour, A., Bakar, F. A., Mohamed, A. K. S., Forghani, B., & Ab-Kadir, M. S. B., et al. (2012). A glutamic acid-producing lactic acid bacteria isolated from Malaysian fermented foods. *International Journal of Molecular Sciences*, *13*(5), 5482–5497.

Zareian, M., Ebrahimpour, A., Sabo Mohamed, A. K., & Saari, N. (2013). Modeling of glutamic acid production by *Lactobacillus plantarum* MNZ. *Electronic Journal of Biotechnology*, *16*(4), 1–16.

Zeng, Q., Gong, J., Liu, X., & Chen, C. (2019). Gut dysbiosis and lack of short chain fatty acids in a Chinese cohort of patients with multiple sclerosis. *Neurochemistry International*, *129*, 104468. doi: 10.1016/j.neuint.2019.104468.

Zhang, C., Brandt, M. J., Schwab, C., & Gänzle, M. G. (2010). Propionic acid production by cofermentation of *Lactobacillus buchneri* and *Lactobacillus diolivorans* in sourdough. *Food Microbiology*, *27*(3), 390–395.

Zhang, Q., Zeng, L., Tan, X., Tang, J., & Xiang, W. (2017). An Efficient γ-aminobutyric acid (GABA) producing and nitrite reducing ability of *Lactobacillus plantarum* BC114 isolated from Chinese paocai. *Food Science and Technology Research*, *23*(5), 749–755.

Zhao, J., Tian, F., Yan, S., Zhai, Q., Zhang, H., & Chen, W. (2018). *Lactobacillus plantarum* CCFM10 alleviating oxidative stress and restoring the gut microbiota in D-galactose-induced aging mice. *Food and Function*, *9*(2), 917–924.

Zommiti, M., Connil, N., Ben Hamida, J., & Ferchichi, M. (2017). Probiotic characteristics of *Lactobacillus curvatus* DN317, a strain isolated from chicken ceca. *Probiotics and Antimicrobial Proteins*, *9*(4), 415–424. http://www.ncbi.nlm.nih.gov/pubmed/28741151 (April 5, 2020).

Chapter 18

Bacteriocins Produced by Probiotic Microorganisms

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1 Introduction

Probiotics are defined by the World Health Organization (WHO) and Food Agricultural Organization (FAO) as nonpathogenic living organisms that benefit host health when they are administered into the organism in sufficient quantities World Gastroenterology Organisation Global Guidelines, 2017. Although *Lactobacillus* and *Bifidobacterium* species are the most known probiotic microorganisms, there are also *Bacillus*, *Saccharomyces*, *Lactococcus*, *Enterococcus*, *Streptococcus*, *Leuconostoc*, *Escherichia*, and *Pediococcus* species with probiotic properties (Fijan, 2014).

An ideal probiotic bacterium must accomplish the subsequent criteria and functional that includes the following (Gupta, Jeevaratnam, & Fatima, 2018; Yangılar, 2015):

1. it must be of human origin;
2. it must be nonpathogenic and nontoxic;
3. it must be resistant to acidic conditions, bile, and pancreatic enzymes;
4. it must be good adhesion to human intestinal epithelial surfaces;
5. it must improve and modulate the immune system;
6. it must be colonized in the digestive and urinary systems;
7. it must produce antimicrobial compounds;
8. it must be cultured on an industrial scale; and
9. it must maintain stability and viability during the manufacturing processes, transportation, and storage.

As a result of clinical studies on probiotics, the results obtained regarding their health benefits can be listed as follows (Vijayaram & Kannan, 2018):

- Probiotics have the ability to lower total cholesterol and low-density lipoprotein levels.
- Probiotics can modulate the immune system.
- Probiotics reduce the production of local proinflammatory cytokines, thereby stabilizing the immunological barrier in the intestinal mucosa, and may benefit in the treatment of inflammatory bowel diseases such as ulcerative colitis, irritable bowel syndrome, Crohn's disease, and pouchitis (Endeshaw, Gizaw, Tsegay, Tefera, & Aynalem, 2018).
- Probiotics can reduce the incidence of colorectal and colon cancer, but limited studies are available on this topic.
- Probiotics may exert antimicrobial effects in the prevention and treatment of common cold, urinary tract, and nosocomial infections.
- Probiotics could possibly reduce the weight gain and improve some metabolic parameters of the associated in obesity (Wang et al., 2019).
- Seven randomized placebo-controlled clinical trials result directly related to the effect of probiotics on metabolic control in type 2 diabetes mellitus highlighted the importance of gut microbiota in diabetes development. As a new strategy in the treatment of diabetes, the effects of probiotics in combination with conventional drugs should be evaluated in clinical studies (Bordalo Tonucci et al., 2017).

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00018-6
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- Although the role of probiotics in the prevention and treatment of allergy is not certain today, there are clinical studies related to their effectiveness in food allergy and atopic dermatitis in the literature (Endeshaw et al., 2018).
- Probiotics are also effective and reliable in the treatment of gastrointestinal system disorders (Anbari, Firouzi, & Abbaszadeh, 2019).

Some *Lactobacillus* and *Bifidobacterium* species and *Saccharomyces boulardii* produce bioactive compounds that have beneficial effects on host health. These compounds are as follows:

1. Amino acids and peptides: tryptophan, tyrosine, arginine, lysine, amino acids metabolites, gamma-aminobutyric acid, Val-Pro-Pro, Ile-Pro-Pro, β-casein f (72–81), and Ser-Lys-Val-Tyr-Pro-Phe-Pro-Gly-Pro-Ile (SLVYPFPGPI) peptides (Linares et al., 2017; Indira, Venkateswarulu, Peele, Bobby, & Krupanidhi, 2019).
2. Vitamins: vitamins B1 (thiamin), B2 (riboflavin), B6 (pyridoxine), B7 (biotin), B9 (folic acid), B12 (cobalamin), and K (Linares et al., 2017).
3. Enzymes: amylases, peptidases, β-galactosidases, casein phosphatase, lysozyme, lactoperoxidase, superoxide dismutase, and catalase (Gupta et al., 2018).
4. Oligosaccharides: inulins and levans.
5. Exopolysaccharides (EPS): EPS are long-chain polysaccharides composed of branched, repeated sugar units or sugar derivatives, produced by bacteria and microalgae. EPS contain varying proportions of sugar units such as glucose, galactose, mannose, rhamnose, *N*-acetylglucosamine, and *N*-acetyl galactosamine. The first bacterial EPS are thought to have been produced by *Leuconostoc mesenteroides* in wine in the mid-19th century (Uchechukwu, Green, & Okoh, 2012).
6. Short-chain fatty acids: lactic acid, 3-phenyllactic acid, 4-hydroxyphenyllactic acid, butyric acid, acetic acid, and propionic acid.
7. Immunomodulatory compounds.
8. Bacteriocins.
9. Others: diacetyl, acetaldehyde, acetoin, hydrogen peroxide, carbon dioxide, reuterin (β-OH-propionic aldehyde), reutericyclin, benzoic acid, methylhydantoin, and mevalonolactone (Suskovic et al., 2010).

In this chapter, bacteriocins produced by probiotic *Lactobacilli* and other probiotic microorganisms, their structural properties and their effects will be discussed.

2 Bacteriocins

In 1925 the substance produced by Andre Gratia with *Escherichia coli* strain 1 against another *E. coli* culture was named as Principe V. In 1946 the term "Colisin" was derived by Gratia and Fredericq. Finally, in 1953, these highly specific antibacterial proteins were named "Bacteriocin" by Jacob et al. In the definition for this term, bacteriocins are very narrow spectrum bactericidal proteins that are adsorbed to specific cell envelope receptors and can be characterized by lethal biosynthesis and are also known as colisins (Tagg, Dajani, & Wannamaker, 1976; Chen & Hoover, 2003).

Due to the resistance to antibiotics and their adverse effects, the beneficial effects of bacteriocins are currently being evaluated in the food industry, human and animal health (Table 18.1). Bacteriocins are used in the food industry as bioprotectors or biopreservatives to extend the shelf life of foods. In animal health, they are utilized for therapeutic or prophylactic purposes for poultry and swine by oral administration of purified or semipurified bacteriocin preparations or by inoculating animal feed with bacteriocin-producing bacteria (Lagha, Haas, Gottschalk, & Grenier, 2017). Recently, detailed studies have been conducted on whether some bacteriocins (plantaricin, nisin, pyocin, colicin, pediocin, and microcin) can be used as an alternative agent in cancer treatment due to their in vitro cytotoxic activity (Cornut, Fortin, & Soulières, 2008; Baindara, Korpole, & Grover, 2018).

Bacteriocins can be synthesized by Gram-negative bacteria such as *E. coli*, *Klebsiella pneumonia*, *Enterobacter cloacae*, *Serratia marcescens*, and *Hafnia alvei*, *Pseudomonas* sp. as well as Gram-positive bacteria such as *Lactobacillus*, *Staphylococcus*, and *Bacillus* species; low-molecular weight antimicrobial peptides with narrow spectrum and lethal effects. When microorganisms producing bacteriocin were examined, it was found that the Archaea synthesized bacteriocin-like antimicrobial peptides, also known as archaeocins. The number of scientific studies on bacteriocins that lactic acid bacteria (LAB) have synthesized is higher than those on other bacteriocins (Güllüce, Karadayı, & Barış, 2013). Among the most important genera of the LAB are *Lactobacillus*, *Lactococcus*, *Carnobacterium*, *Enterococcus*, *Lactosphaera*, *Leuconostoc*,

TABLE 18.1 Comparison of bacteriocins and antibiotics.

Properties	Bacteriocin	Antibiotic	References
Applications	Food, livestock health, and medicine	Food, livestock health, and medicine	Bemena, Mohamed, Fernandes, and Lee, (2014)
Biosynthesis	Ribosomal	Enzymes	Bemena et al. (2014)
Production	Primary metabolism	Secondary metabolism	Faustino Jozala et al. (2015)
Bioactivity spectrum	Narrow	Broad	Dever and Dermody (1991)
Active pH range	Broad	Narrow	Bemena et al. (2014)
Thermostability	High	Low	Dever and Dermody (1991)
Resistance development mechanism	1. Modifies in cell membrane fatty acids and receptors 2. D-Alanylation of cell wall teichoic acids 3. L-Lysinylation of cell membrane phospholipids	1. Enzymatic degradation 2. Changes in bacterial proteins 3. Alterations in membrane permeability	Dever and Dermody (1991)
Mode of action	Increasing the permeability of the cell membrane, formation of pore, blocking the function of DNA, and RNA	Cell wall, nucleic acid and protein synthesis inhibition, degradation in plasma membrane	Faustino Jozala et al. (2015)
Toxicity	Currently unknown	Available	Dever and Dermody (1991)
Antibiofilm features	Strong	Resistance	Meade et al. (2020)
Oral bioavailability	Weak	Potent	Meade et al. (2020)
Effective concentration range	Pico-nanomolar	Micromolar	Meade et al. (2020)

Melissococcus, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella* (Parada, Ricoy, Medeiros, & Soccol, 2007).

3 Gram-negative bacteriocins

(Kortel & Moreno, 1992) classified bacteriocins synthesized by Gram-negative microorganisms as lantibiotics, heat-resistant nonlantibiotics, colicins, and microcins. Bacteriocins were named based on the producer species at first. The molecular size of the microcins is less than 10 kDa, and the colicins are larger than 10 kDa. Colicin, isolated from *E. coli* and the most studied, is the first Gram-negative bacteriocin. Subsequent research has shown that many Gram-negative bacteria, in which bacteriocins are not only produced by *E. coli*, also produce choline-like bacteriocin. Alveicins of *H. alvei*, cloacins of *E. cloacae*, Klebicins of *K. pneumonia*, marcescens of *S. marcescens*, and pyocins of *Pseudomonas* sp. are important examples representing bacteriocins produced by Gram-negative bacteria (Güllüce et al., 2013).

In a review by Cesa-Luna et al. (2020), Gram-negative bacteriocins are classified into the following five main groups:

- Colicins: bacteriocins produced by *E. coli* and whose molecular weight is greater than 10 kDa.
  - Subclass I: cause pore formation in the bacterial cell wall.
  - Subclass II: break down DNA and RNA.
- Colicin-like: bacteriocins produced by other bacteria than *E. coli* and whose molecular weight is greater than 10 kDa.
  - Subclass I: cause pore formation in the bacterial cell wall.
  - Subclass II: break down DNA and RNA.
- Microcins: molecular weights of this class of bacteriocins are less than 10 kDa. They exert antibacterial action against target cells via pore formation, DNase and RNase function, and inhibition of protein synthesis.
  - Subclass I: the molecular weights of these bacteriocins are less than 5 kDa and posttranslationally modified [microcins B17, C (or C7–C51), and J25].
  - Subclass II: the molecular weights of these bacteriocins are between 5 and 10 kDa and unmodified or partially modified (microcins L, V, and N) (Rebuffat, 2012; Simons, Alhanout, & Duval, 2020).

- Klebicins
- Pyocins
  - R type
  - S type
  - F type
- Tailocins

Colicin-like bacteriocins are plasmid-encoded antimicrobial peptides produced by *E. coli* and other members of the Enterobacteriaceae family. The molecular weights of these bacteriocins range from 10 to 80 kDa and have the ability to inhibit *Salmonella* and other *E. coli* strains. Colicin and colicin-like bacteriocins consist of three functional domains; the N-terminal translocation domain that regulates the target and binds to the receptor on the sensitive cell, the central receptor-binding domain that attaches to target cell outer membrane receptor, and the C-terminal killing domain that acts as nuclease to break down target DNA and RNA.

Bacteriocins produced by *Klebsiella* strains were first described by Hamon and Peron in 1963 (Kareem & Al-Salmani, 2017). Although these bacteriocins are also known as klebocin, the term klebicin is more preferred. Klebicines produced by *Klebsiella* species bearing klebocinogenic plasmid are organized into three functional domains (central, N-terminal, and C-terminal domains). This bacteriocin group binds to the receptors of the target cells that provide the entry of nutrients. They are then translocated through the cell envelope and pass through the periplasm with the Tol and TonB systems. At the end of this process, klebicins cause cell death by forming a voltage-dependent channel in the inner membrane or by breaking down DNA, tRNA (klebicin D), or rRNA (klebicins B and C) with their endonuclease activities (Ghazaryan, 2013).

Pyocins produced by *Pseudomonas aeruginosa* were isolated by Jacob in 1954. Pyocins are classified as R, S, and F and purified. It has been determined that R and F types are resistant to trypsin enzyme and S type is sensitive to proteinase and trypsin enzymes (Jacob 1954; Al-Rubiee, Al-Mudaffar, Hassan, & Al-ani, 1988). The studies conducted in the following years provided more detailed information about the structures of R-, S-, and F-type pyocins. R- and F-type pyocins are structurally similar to each other. Both have high molecular weight and bacteriophage tails. When evaluated in terms of bacteriophage tails, R-type pyocins are rigid and contractile, while F-type pyocins are flexible and noncontractile rodlike. Pyocins exert their antibacterial effects through receptors on the target cell. Although the R type is resistant to nuclease–protease enzymes, their mechanism of action is the depolarization of the cytoplasmic membrane by pore formation (Fischer et al., 2012). S-type pyocins are protease-sensitive, soluble, and small molecular weight proteins. These pyocins consist of two components. One of these components is the large protein part, which performs the killer activity by causing chromosomal DNA degradation as a result of endonuclease activity localized at the C-terminal. The smaller component acts as an immunity protein. This component is capable of forming pores and breaking down DNA and causes cell death through these mechanisms (Duport, Baysse, & Michel-Briand, 1995; Michel-Briand & Baysse, 2002).

Bacteriocins of tailocins group are of high molecular weight, thermolabile, and resistant to proteases and also known as phage taillike bacteriocins. These bacteriocins are morphologically divided into two groups. First group R-type tailocins (myotailocins) are rigid rodlike sheathed particles. Second group F-type tailocins (siphotailocins) are flexible sheathless particles (Dorosky, Yu, Pierson, & Pierson, 2017; Cesa-Luna et al., 2020). Tailocins bind to specific cell receptors, ensuring that the cytoplasmic membrane is depolarized. Then macromolecule synthesis is inhibited and cell death occurs. For example, tailocin (BceTMilo) produced by *B. cenocepacia* BC0425 is claimed to be adsorbed by binding to a D-glucose receptor on the *P. aeruginosa* cell surface.

4 Gram-positive bacteriocins

Gram-positive bacteriocins are generally cationic, amphiphilic, and membrane-permeabilizing proteins with a molecular weight less than 8 kDa. There are differences between the critical parameters considered in the classification of Gram-positive bacteriocins, and there are still conflicts of opinion in this context (Maqueda et al., 2008).

Bacteriocins were divided into four classes by Klaenhammer (1993), according to their mechanism of action, molecular weight, thermal resistance, enzymatic sensitivity, amino acid sequence, and the presence of modified amino acid (Fig. 18.1). Therefore three main classification systems used between 1993 and 2016 will be mentioned in the classification of bacteriocins.

Cotter, Hill, and Ross (2005) have modified the classification of LAB bacteriocins and divided it into the following two main categories: lantibiotics (class I) and nonlanthionine bacteriocins (class II) (Fig. 18.2) (Cotter et al., 2005). Class III in the classification of Klaenhammer was named Bacteriolysin by Cotter. Class IV bacteriocins are also canceled because they contain complex lipid and carbohydrate moieties (Dinçer, Kıvanç, & Karaca, 2010).

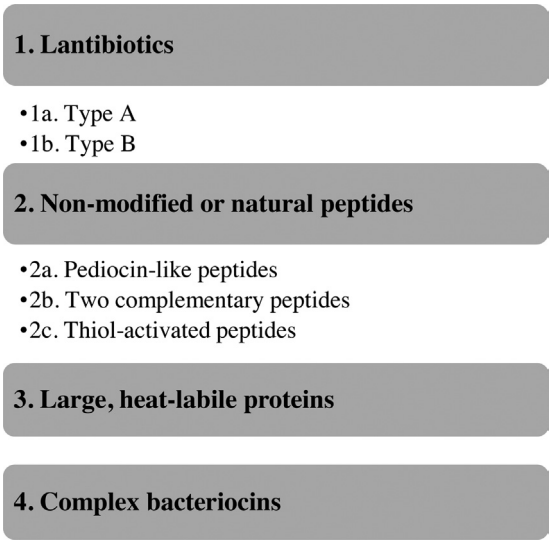


FIGURE 18.1 Bacteriocin classification by Klaenhammer (1993).

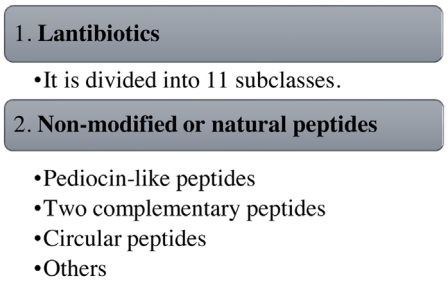


FIGURE 18.2 Bacteriocin classification by Cotter et al. (2005) and Dinçer et al. (2010).

In the article published by Kumariya et al. (2019), bacteriocins are classified under three main headings. This classification system is cited from the article published by (Liu, Pnag, Zhang, & Cai, 2014).

4.1 Class I bacteriocins (modified peptides)

Class I bacteriocins are ribosomally synthesized, noncationic, pore-forming, thermostable, low-molecular weight, and linear or globular peptides (<5 kDa) containing 19–50 amino acids (Yang, Lin, Sung, & Fang, 2014). Nonstandard amino acids such as labyrinthine, lanthionine, β-methyllanthionine, dehydrobutyrine, and dehydroalanine are formed as a result of post-translational modification with enzymatic reactions of class I bacteriocins. Additionally, they exert antibacterial activity against Gram-positive bacteria, while interestingly, they do not inhibit Gram-negative bacteria. Class I bacteriocins are also divided into three subgroups: class Ia (Lantibiotics), class Ib (Labyrinthopeptins), and class Ic (Sanctibiotics) (Table 18.2).

4.2 Class II bacteriocins (unmodified peptides)

Class II bacteriocins are thermostable, cationic, unmodified, hydrophobic, and nonlanthionine containing membrane active peptides (<10 kDa) containing 30–70 amino acids. They are divided into four subgroups: class IIa (pediocin-like bacteriocins), class IIb (two-peptides unmodified bacteriocins), class IIc (circular bacteriocins), and class IId (nonpediocin-like linear bacteriocins) (Table 18.3). Class IIa bacteriocins exert antilisterial activity (Meade, Slattery, & Garvey, 2020).

4.3 Class III bacteriocins (large proteins)

Bacteriocins of this group are hydrophilic, thermolabile, and high-molecular weight (>30 kDa) peptides. Class III bacteriocins are divided into two subgroups: class IIIa (bacteriolysins) and class IIIb (nonlytic bacteriocins) (Table 18.4) (Ramu et al., 2015).

TABLE 18.2 The class I bacteriocins and their host producers.

Bacteriocins	Examples	Host producers	References
Class Ia	Epidermin	*Staphylococcus epidermidis*	Kumariya et al. (2019)
	Gallidermin	*Staphylococcus gallinarum*	Tagg et al. (1976)
	Lacticin 481	*Lactococcus lactis*	Tagg et al. (1976)
	Lactocin S	*Lactobacillus sakei*	Tagg et al. (1976)
	Mutacin II	*Streptococcus mutans*	Kodali, Lingala, Karlapudi, Venkateswarulu, and Dulla (2013)
	Nisin	*L. lactis*	Gomes, Rodrigues, Winkelstroter, Nomizo, and De Martinis (2012)
	Pep5	*S. epidermidis*	Meade et al. (2020)
	Plantaricin W	*Lactobacillus plantarum*	Kodali et al. (2013)
	Staphylococcin C55	*Staphylococcus aureus*	Kodali et al. (2013)
	Streptococcin A-FF22	*Streptococcus pyogenes*	Kodali et al. (2013)
	Subtilin	*Bacillus subtilis*	Meade et al. (2020)
Class Ib	Actagardin	*Actinoplanes* ssp.	Tagg et al. (1976)
	Ancovenin	*Streptomyces* spp.	Tagg et al. (1976)
	Cinnamycin	*Streptomyces cinnamoneus*	Tagg et al. (1976)
	Duramycin	*S. cinnamoneus*	Tagg et al. (1976)
	Labyrinthopeptin A1	*Actinomadura namibiensis*	Kumariya et al. (2019)
	Lacticin 3147A	*L. lactis* DPC3147	Gomes et al. (2012)
	Mersacidin	*B. subtilis*	Osama (2019)
Class Ic	Thuricin CD	*Bacillus thuringiensis*	Kumariya et al. (2019)
	Plantaricin C	*L. plantarum* LL441	Kumariya et al. (2019)

TABLE 18.3 The class II bacteriocins and their host producers.

Bacteriocins	Examples	Host producers	References
Class IIa	Acidocin A	*Lactobacillus acidophilus* TK 9201	Cintas, Casaus, Herranz, Nes, and Hernández (2001)
	Bavaricin A	*Lactobacillus bavaricus* MI401	Cintas et al. (2001)
	Carnobacteriocin BM1	*Chryseobacterium piscicola* LB17B	Cintas et al. (2001)
	Carnobacteriocin B2	*C. piscicola* LV17B	Cintas et al. (2001)
	Curvacin A	*Lactobacillus curvatus*	Cintas et al. (2001)
	Divercin V41	*Carnobacterium divergens*	Tagg et al. (1976)
	Enterocin A	*Enterococcus fecium*	Tagg et al. (1976)
	Enterocin NKR-5-3	*E. fecium* NKR-5-3	Meade et al. (2020)
	Lactococcin MMFII	*Lactococcus lactis*	Tagg et al. (1976)
	Leucocin A	*Leuconostoc gelidium*	Cintas et al. (2001)
	Leucocin A-UAL 187	*Leuconostoc gelidum*	Tagg et al. (1976)
	Mesentericin Y105	*Leuconostoc mesenteroides* Y105	Tagg et al. (1976)
	Mesentericin 52B	*L. mesenteroides* FR52	Cintas et al. (2001)
	Mesentericin B105	*L. mesenteroides* Y105	Cintas et al. (2001)
	Microcin L	*Escherichia coli*	Meade et al. (2020)
	Microcin N/24	*E. coli*	Meade et al. (2020)
	Pediocin PA-1/AcH	*Pediococcus pentosaceus*, *Pediococcus acidilactici*	Kumariya et al. (2019)
	Sakacin A	*L. sakei* LB706	Cintas et al. (2001), Kumariya et al. (2019)
	Sakacin P	*L. sakei* LTH673	Cintas et al. (2001), Kumariya et al. (2019)
	Sakacin 674	*L. sakei* LB674	Cintas et al. (2001)
	Sakacin 1	*L. sakei* 1	Gomes et al. (2012)

TABLE 18.3 The class II bacteriocins and their host producers. (*Cont.*)

Bacteriocins	Examples	Host producers	References
Class IIb	Acidocin J1132 (α, β)	*L. acidophilus* JCM1132	Cintas et al. (2001)
	Enterocin 1071	*Enterococcus fecalis* FAIR-E 309	Kumariya et al. (2019)
	Enterocin NKR-5-3AZ	*E. fecium* NKR-5-3AZ	Meade et al. (2020)
	Lactococcin G	*L. lactis* LMG2081	Cintas et al. (2001)
	Lactococcin M	*L. lactis*, *Lactococcus cremoris* 9B4	Tagg et al. (1976), Cintas et al. (2001)
	Lactacin F	*Lactobacillus johnsonii*	Tagg et al. (1976)
	Leucocin H	*Leuconostoc* sp. MF215B	Cintas et al. (2001)
	Microcin M	*E. coli*	Meade et al. (2020)
	Microcin H47	*E. coli*	Meade et al. (2020)
	Plantaricin A	*L. plantarum*	Tagg et al. (1976)
	Plantaricin S	*L. plantarum* LCPO10	Tagg et al. (1976), Cintas et al. (2001)
	Plantaricin EF	*L. plantarum* C11	Tagg et al. (1976), Cintas et al. (2001)
	Plantaricin JK	*L. plantarum* C11	Tagg et al. (1976), Cintas et al. (2001)
	Thermophilin 13 (ThmA/ThmB)	*Streptococcus thermophilus* SPi13	Cintas et al. (2001)
Class IIc	Acidocin B	*L. acidophilus* M46	Tagg et al. (1976), Cintas et al. (2001)
	Bacteriocin 31	*E. fecalis* YI17	Cintas et al. (2001)
	Carnobacteriocin A	*Carnobacterium piscicola* LV17A	Tagg et al. (1976), Oscáriz and Antonio (2001)
	Carnocyclin A	*Carnobacterium maltaromaticum* UAL307	Kumariya et al. (2019)
	Cerein 7/8	*Bacillus cereus* Bc7	Oscáriz and Antonio (2001)
	Divergicin A	*C. divergens* LV13	Tagg et al. (1976), Cintas et al. (2001)
	Enterocin AS-48	*E. fecalis*	Cintas et al. (2001)
	Enterocin B	*E. fecium* T136, *E. fecium* CECT492	Tagg et al. (1976), Oscáriz and Antonio (2001)
	Enterocin EJ97	*E. fecalis* EJ97	Tymoszewska, Diep, Wirtek, and Aleksandrzak Piekarczyk (2017)
	Enterocin P	*E. fecium*	Tagg et al. (1976)
	Gassericin A	*Lactobacillus gasseri*	Kumariya et al. (2019)
	Garvicin ML	*Lactococcus garvieae*	Kumariya et al. (2019)
	Lactococcin 972	*L. lactis* IPLA972	Cintas et al. (2001)
	Lactococcin B	*L. lactis* subsp. *cremoris* 9 B4	Meade et al. (2020)
	Listeriocin 743A	*Listeria innocua*	Kodali et al. (2013)
Class IId	Acidocin 8912	*L. acidophilus* TK8192	Cintas et al. (2001)
	Bactofencin A	*Lactococcus salivarius*	Kumariya et al. (2019)
	Diacetin B	*L. lactis* subsp. *diacetylactis* UL720	Cintas et al. (2001)
	Divergicin 750	*C. divergens* 750	Cintas et al. (2001)
	Enterocin I	*E. fecium*	Kodali et al. (2013)
	Enterocin L50	*E. fecium* L50	Cintas et al. (2001)
	Enterocin Q	*E. fecium* L50	Cintas et al. (2001)
	Epidermicin N101	*S. epidermidis*	Kumariya et al. (2019)
	Gassericin B3	*L. gasseri* HCM2124	Cintas et al. (2001)
	Garvicin Q	*L. garvieae* BCC 43578	Tymoszewska et al. (2017)
	Lactobin A	*Lactobacillus amylovorus* LMG P-13139	Cintas et al. (2001)
	Lactocin 705	*Lactobacillus casei* CRL705	Cintas et al. (2001)
	Lactococcin A	*L. lactis* LMG2130	Cintas et al. (2001), Oscáriz and Antonio (2001)
	Leucocin B-TA33a	*L. mesenteroides* TA33a	Cintas et al. (2001)
	Leucocin N	*L. mesenteroides* QU 15	Meade et al. (2020)
	LsbB	*L. lactis* subsp. *lactis*	Kumariya et al. (2019)

TABLE 18.4 The class III bacteriocins and their host producers.

Bacteriocins	Examples	Host producer	References
Class IIIa	Enterolysin A	*Enterococcus fecalis*	Kumariya et al. (2019)
	Helveticin V-1829	*Lactobacillus helveticus*	Tagg et al. (1976)
Class IIIb	Caseicin 80	*Lactobacillus casei* B80	Cintas et al. (2001)
	Helveticin J	*L. helveticus* 481	Tagg et al. (1976), Cintas et al. (2001)
	Lysostaphin	*Staphylococcus simulans* subsp. *staphylolyticus*	Ramu et al. (2015)
	Streptococcin A-M57	*Streptococcus pyogenes*	Kumariya et al. (2019)

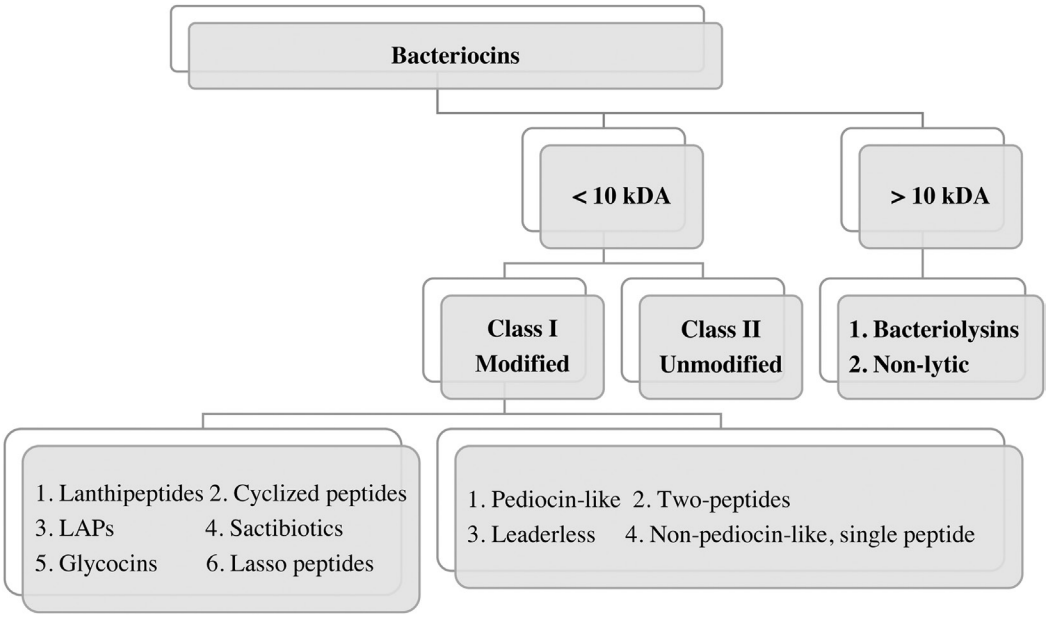


FIGURE 18.3 Bacteriocin classification by Alvarez-Sieiro et al. (2016).

Alvarez-Sieiro, Montalbán-López, Mu, and Kuipers (2016) have proposed a new classification system based on the biosynthesis mechanism and biological activities, taking into account the classification systems made up to that date. New ribosomally synthesized and posttranslationally modified peptides subgroups, which are produced by bacteria and do not meet the previous classifications, despite being compatible with the definition of bacteriocin, have been discovered. Based on the biosynthesis mechanism and biological activities, the classification of bacteriocins produced by LAB has been modified with the addition of some subclasses (Fig. 18.3) (Alvarez-Sieiro et al., 2016).

5 The mechanism of antibacterial activity of bacteriocins

Before talking about the antibacterial mechanism of bacteriocin, it is necessary to briefly mention the mechanism of action of antibiotics. Conventional antibiotics exert their antibacterial effects with the following six main mechanisms: (1) cell-wall synthesis inhibition, (2) protein synthesis inhibition, (3) DNA synthesis inhibition, (4) RNA synthesis inhibition, (5) mycolic acid synthesis inhibition, and (6) folic acid synthesis inhibition. As a result of scientific researches conducted to date, bacteriocins have been found to have antibacterial effects with all other mechanisms except folic acid synthesis inhibition (Cotter, Ross, & Hill, 2013). A vast of bacteriocins inhibit only by destroying the electrolytic charge balance of the cell membrane. Since bacteriocins released by some LAB are positively charged molecules, they act on bacterial cytoplasmic membrane. Electrostatic interaction occurs under the action of negatively charged components (teichoic acids, teichuronic and lipoteichoic acids, and acidic polysaccharides or phospholipids) in the cell membrane, and as a result bacteriocins attach to the cell membrane. At the end of the reaction, the hydrophobic part of the bacteriocin enters the membrane structure and causes pore formation.

Bacteriocins (class IIa) with the YGNV sequence in the N-terminal regions display a noticeable antilisterial selectivity and form ion channels on the membrane, thereby causing ions such as magnesium and potassium to leak out. Energy production is prevented by the impairment of membrane permeability and depletion of proton motive force of bacteria, and consequently protein and nucleic acid synthesis is inhibited (Oscáriz & Antonio, 2001; Çayır Üstündağ & Yalçın, 2017).

Lantibiotics target lipid II that intermediates the biosynthesis of peptidoglycans found in the bacterial cell wall. And in this way, they can display antibacterial effects by inhibiting peptidoglycan synthesis (Punyauppapath, Phumkhachorn, & Rattanachaikunsopon, 2015).

The mannose phosphotransferase system (Man-PTS) on sensitive cells, which is an important sugar intake system in Gram-positive and Gram-negative bacteria, can be used as receptor molecules by some bacteriocins. The Man-PTS include four units: IIA, IIB, IIC, and IID. Only membrane proteins IIC and IID are target receptors for class IIa bacteriocins (pediocin PA-1, enterocin A, enterocin P, and sakacin P), class IId bacteriocins (garvicin Q, lactococcin A, and B), and microcin E492 synthesized from *Klebsiella* species. Some class IIa bacteriocins structurally contain an N-terminal β-sheet unit and a C-terminal α-helix containing hairpin or hairpin-like units. First, the β-sheet unit of the bacteriocin binds to the extracellular loop of IIC and then the hairpin unit of the bacteriocin connects to the transmembrane helices of the Man-PTS. These successive reactions cause conformational changes in Man-PTS proteins. These changes lead to uncontrolled flow of essential molecules, impaired membrane integrity, and eventually cell death (Swe, Cook, Tagg, & Jack, 2009; Kjos, Nes, & Diep, 2011; Hassan, Kjos, Nes, Diep, & Lotfipour, 2012).

The newest recently identified target for bacteriocins is the inhibition of septum formation which is in development of mucopeptide layers and cytoplasmic membranes during mitotic division. Bacterial cell division is known as the process of producing two living descendants from a progenitor cell. This includes two major coordinated steps: the replication and separation of the bacterial chromosome and division of the progenitor cell by cytokinesis, also defined as the formation of septum in bacteria. During cytokinesis, bacteriocins play a role in the formation of a bulge and termination of the cell cycle, causing bacterial inactivation (Maryam, Maqsood, & Farooq, 2017).

As a result, we can summarize the antibacterial mechanisms of bacteriocins as follows:

- inhibition of cell wall biosynthesis (NAI-107, nisin A, and nukacin ISK-1);
- inhibition of DNA replication and transcription (microcin B17, colicins, and carocin S2);
- septum formation (garvicin A, lactococcin 972);
- inhibition of protein synthesis (colicins E3-E6, cloacin DF13, etc.); and
- membrane disturbance (geobacillin I, bac-GM17, plantaricin J/K, plantaricin E/F, dysgalacticin, lactococcin, pediocin-like bacteriocins, mesentericin Y105, nisin A, and lacticin Q) (Cavera, Arthur, Kashtanov, & Chikindas, 2015).

6 Applications of bacteriocins

The applications of LAB (*Lactobacillus*, *Streptococcus*, *Enterococcus*, *Leuconostoc*, *Pediococcus*, and *Bifidobacterium* sp.), which are the most commonly used probiotics, in food industry and human health will be mentioned.

6.1 Application in foods

Nonpathogenic microorganisms or their metabolites used to increase safety and extend the shelf life of foods are defined as bioprotectors. Bacteriocins, especially produced by LAB, are used as bioprotectors in foods because they are Generally Recognized as Safe, tolerant to pH and temperature, and are not active or toxic to eukaryotic cells (Dinçer et al., 2010). Bacteriocins or protective cultures can be added directly to food, or bacteriocins alone can also be applied to food packaging materials. For these purposes, LAB are used as protective cultures, while nisin, which is allowed for legal use, is used as bacteriocin (Kurt & Zorba, 2005).

The benefits of bacteriocins added to foods as bioprotectors are as follows:

- The shelf life of foods is extended.
- Extra protection is provided at temperatures under storage conditions.
- The use of chemical preservatives can be reduced.
- Due to their peptide and protein structure, they can be affected by gastric secretions with proteolytic enzymes of pancreatic origin, so they can be digested in the body.
- The risk of distribution of foodborne pathogens with the food chain may decrease.

Disadvantages that may be encountered in the use of bacteriocins as bioprotectors in foods are as follows:

- limited activity spectrum,
- interaction with food ingredients,
- development of bacteriocin-resistant bacteria,
- limited diffusion in solid matrices, and
- inactivation with proteolytic enzymes.

It is reported that many purified/semipurified bacteriocins or bacteriocin-producing LAB are utilized in the control of pathogens especially in milk and dairy products, meat and poultry products, vegetable-based foods and drinks, and fish and seafood products.

Nowadays, nisin produced by *Lactococcus lactis* is the most studied bacteriocin, and there are regulations on its use in over 50 countries around the world (Faustino Jozala, De Lencastre Novaes, & Pessoa Junior, 2015). The action spectrum of nisin is wider compared to many other bacteriocins, and they are highly active for acidic foods and against Gram-positive microorganisms. The solubility of nisin at pH 2 is 228 times higher than pH 8. Therefore its solubility decreases as the pH increases. The FAO/WHO organization allowed the use of nisin as a food additive in 1969 and is used safely and effectively in many countries of the world. For the first time, it has been permitted in cream cheeses to be used as a protective additive. The US Food and Drug Administration has set the daily acceptable amount of nisin for adults to be 2.9 mg.

In addition to nisin, different bacteriocins are also used in foods. Pediocin AcH (Munster cheese, chicken meat, and chicken meat sausage), Enterocin 4 (Manchego cheese), Linocin M-18 (in red cheese production), Piscicolin 126 (ham), Leucocin A (beef), Lactocin 705 (minced beef), Enterocin (ham, pork, chicken breast, and sausage), and Pediocin PA-1 (fermented sausage) are examples (Kurt & Zorba, 2005).

6.2 Animal health

Bacteriocins are also important in terms of animal health. Mastitis is the biggest problem encountered especially in cattle during breastfeeding. In this case, antibiotics are usually used in treatment. But, as is known, some of the antibiotics pass into the milk during lactation period and decrease the quality of milk. In addition, as a result of the consumption of milk with antibiotics, resistance to this antibiotic may develop. Therefore researches have been carried out considering that bacteriocins can be used in treatment instead of antibiotics. As a result of researches, it has been found that Lacticin 3147 produced by the strain of *L. lactis* DPC3147 inhibits *Bacillus* sp., *Enterococcus* sp., *Lactobacillus* sp., *Pediococcus pentriceans*, *Streptococcus aureus*, and *Streptococcus thermophilus* strains. Ryan, Meaney, Ross, and Hill (1998) determined that Lacticin 3147 has a bactericidal effect against strains of *Staphylococcus* and *Streptococcus* causing mastitis by agar diffusion method (Ryan et al., 1998).

6.3 Human health

6.3.1 Antiviral activity

Since viral diseases are very lethal and viruses are mutated, the development of new, effective, inexpensive, and highly bioavailable antiviral drugs is an important strategic goal. In this context, antiviral activity studies are also carried out on bacteriocins.

Bacteriocin bacST284BZ produced by the strain of *Lactobacillus paracasei* ST284BZ inhibits the *Herpes simplex* virus (Todorov et al., 2008). On the other hand, Quintana et al. (2014) examined the antiviral effects of subtilosin from *Bacillus amyloliquefaciens* using Vero cells against *Herpes simplex* type II, known as the cause of genital ulcer disease. The results showed that subtilosin does not protect cells against *Herpes simplex* virus II, but it strongly inhibits both extracellular and total virus production after infection (Quintana et al., 2014; Férir et al., 2013).

Labyrinthopeptin A1 (LabyA1) isolated from the actinomycete *Actinomadura namibiensis* DSM 6313 has been tested for both HIV and Herpes Simplex Virus for its antiviral effect. LabA1 (EC_{50s}: 0.70–3.3 mM) shows consistent anti-HIV effect on MT-4, peripheral blood mononuclear cells, and Monocyte-derived macrophages cell lines. Compared to HIV, LabyA1 (EC_{50s}: 0.29–2.8 μM) showed stronger effects against various Herpes Simplex Virus clinical isolates against various Herpes Simplex Virus clinical isolates (Férir et al., 2013).

Bacteriocins isolated especially from *Enterococcus* strains were found to be effective against Herpes Simplex Virus. Enterocin ST5Ha from *Enterococcus fecium* ST5Ha, Enterocin ST4VV-1 from *Enterococcus mundtii* ST4V, Enterocin CRL35 from *E. mundtii* CRL35, and Enterocin NKR-5-3C from *E. fecium* NKR-5-3 are the examples of bacteriocins (Al Kassaa, Hober, Hamze, Chihib, & Drider, 2014).

6.3.2 Anticancer effect

In vitro and in vivo studies have been conducted by various groups to examine the anticancer effects of bacteriocins. Different cell lines and MTT method were generally used to evaluate the anticancer effects.

Kaur, Balgir, Mittu, Kumar, and Garg (2013b) examined the effect of Fermenticin HV6b produced by *Lactobacillus fermentum* HV6b MTCC10770 on proliferation of hepatocarcinoma (HepG2), perpetual cervical carcinoma (HeLa ATCC CCL2), spleen lymphoblast (Sp2/0-Ag14 ATCC-CRL-1581), kidney embryonal (HEK293 CRL-1573), and breast carcinoma (MCF7 ATCC-HTB-22) cell lines with MTT assay. Cytotoxic activity results indicated that MCF-7 and HEK-293 cell lines showed a slight sensitivity to bacteriocin, while Fermenticin HV6b had a very strong effect in HeLa, Sp2/O, and HepG2 cell lines (Kaur et al., 2013b). Studies on the anticancer effects of bacteriocin are presented in Table 18.5.

TABLE 18.5 Effects of bacteriocins on different cancer cell lines.

Bacteriocin	Host producer	Cell line	References
Azurin	*Pseudomonas aeruginosa*	J774, MCF-7, UISO-Mel-2, U2OS, HL60, K562, HUVEC, HCT-116, MDA-MB-231	Baindara et al. (2018)
Colicin E3	*E. coli*	P388, HeLa, HS913T, V79, BM2	Kaur and Kaur (2015)
Colicin A	*E. coli*	HS913T, SKUT-1, BT474, ZR75, SKBR3, MRC5	Kaur and Kaur (2015)
Colicin E1	*E. coli*	MCF7, HS913T, BM2	Kaur and Kaur (2015), Baindara et al. (2018)
Colicin N	*E. coli*	H460	Ecoy, Chaotham, Suksomtip, Chanchavarote, and Arunmanee (2018)
Nisin A	*Lactococcus lactis* subsp. *lactis*	UM-SCC-17B, UM-SCC-14A, HSC-3, MCF-7, HepG2, Jurkat	Karpinski and Adamczak (2018)
Nisin ZP	*L. lactis* subsp. *lactis*	UM-SCC-17B, HSC-3	Karpinski and Adamczak (2018)
Nisin	*L. lactis* subsp. *lactis*	SW1088	Zainodini et al. (2018)
Plantaricin A	*L. plantarum*	Jurkat, GH4, Reh, PC12, N2A	Kaur and Kaur (2015), Karpinski and Adamczak (2018)
Laterosporulin10 (LS10)	*Brevibacillus* sp. strain SKDU10	MCF-7, HEK293T, HT1080, HeLa, H1299	Baindara, Gautam, Raghava, and Korpole (2017)
Pediocin PA-1	*Pediococcus acidilactici* PAC1.0	A549, DLD-1, HT29, HeLa	Kaur and Kaur (2015), Baindara et al. (2018)
Pediocin CP2	*P. acidilactici* MTCC 5101	MCF-7, HepG2, HeLa, Sp2/0-Ag 14	Karpinski and Adamczak (2018), Baindara et al. (2018)
Pediocin K2a2-3	*P. acidilactici* K2a2-3	HT29, HeLa	Kaur and Kaur (2015), Karpinski and Adamczak (2018)
Pep27 anal2	*Streptococcus pneumoniae*	AML-2, HL-60, Jurkat, SNU-601, MCF-7	Baindara et al. (2018)
Bovicin HC5	*Streptococcus bovis* HC5	MCF-7, HepG2	Karpinski and Adamczak (2018), Baindara et al. (2018)
Microcin E492	*Klebsiella pneumoniae* RYC492	HeLa, Jurkat, Ramos, RJ2.25, KG-1, human colorectal carcinoma cells	Kaur and Kaur (2015), Karpinski and Adamczak (2018), Baindara et al. (2018)
Pyocin S2	*P. aeruginosa* 42A	HepG2, Im9, HeLa, AS-II, mKS-A TU-7, HFFF	Kaur and Kaur (2015), Baindara et al. (2018)
Smegmatocin	*Mycobacterium smegmatis* 14468	HeLa AS-II, HGC-27 mKS-A TU-7	Kaur and Kaur (2015)

A549, Adenocarcinomic human alveolar basal epithelial cells; *AML-2*, acute myeloid leukemia-2; *AS-II*, derived from embryonal carcinoma of ovary; *BM2*, human breast cancer; *BT474*, human mammary–invasive ductal carcinoma; *DLD-1*, colorectal cell line; *GH4*, rat pituitary tumor; *H1299*, human nonsmall cell lung carcinoma cell line derived from the lymph node; *H460*, human lung cancer; *HCT-116*, human colon carcinoma; *HEK293T*, a mutant version of the SV40 large T antigen; *HeLa*, cervical adenocarcinoma; *HepG2*, liver hepatocellular carcinoma; *HFFF*, human fetal foreskin fibroblast cells; *HGC 27*, human gastric carcinoma cell line; *HL-60*, myeloid cell line; *HS913T*, cellosaurus cell line; *HSC-3*, human tongue squamous carcinoma cell line; *HT1080*, fibrosarcoma cell line; *HT29*, colon adenocarcinoma; *HUVEC*, human umbilical vein endothelial cell; *Im9*, human, lymphoblastoid B cell, multiple myeloma; *J774*, mouse macrophage cell line; *Jurkat*, acute T-cell leukemia; *K562*, human immortalized myelogenous leukemia cell line; *KG-1*, human myeloid leukemia cell line; *MCF-7*, mammary gland adenocarcinoma; *MDA-MB-231*, epithelial, human breast cancer cell line; *Mel 2*, human melanoma cell; *mKS-A TU-7*, simian virus 40-transformed BALB/c mouse cells; *MRC5*, diploid human cell culture line composed of fibroblasts; *N2A*, mouse neuroblastoma cell line; *P388*, mouse leukemia cells; *PC12*, a mixture of neuroblastic cells and eosinophilic cells; *Ramos*, Burkitt's lymphoma cell line; *Reh*, non-T, non-B acute lymphoblastic leukemia cell line; *RJ2.25*, B-lymphoblastoid cells; *SKBR3*, human breast cancer cell line; *SKUT-1*, human uterine leiomyosarcoma cell line; *SNU-601*, human stomach carcinoma; *Sp2/0-Ag 14*, spleen lymphoblast; *SW1088*, colorectal cancer cell line; *UM-SCC-14A*, head and neck squamous cell carcinoma; *UM-SCC-17B*, squamous cell carcinoma; *V79*, Chinese hamster lung fibroblast; *ZR75*, human breast cancer cell line.

6.3.3 Antiulcerogenic effect

Bacteriocins (nisin A, lacticins A164, BH5, JW3, NK24, pediocin PO2, and leucocin K) produced by LAB were tested in *Helicobacter pylori* strains by broth microdilution method. As a result of the study, it was found that lactis A164 and lactis BH5 exerted strong antibacterial activity against *H. pylori* strains compared to other bacteriocins (Kim et al., 2003). It has been suggested that the partially purified Bacteriocin BA28 produced by *Pediococcus acidilactici* BA28 is effective against *H. pylori* as a result of preliminary screenings and can be used in the production of new therapeutics for this purpose (Kaur & Garg, 2013). Lim (2015) investigated the antimicrobial effects of purified bacteriocins isolated from *Lactobacillus brevis* BK11 and *Enterococcus fecalis* BK61 on *H. pylori*. It was found that bacteriocins, which were obtained by purification from *L. brevis* BK11 and *E. fecalis* BK61 and molecular weights of which were determined as approximately 6.5 and 4.5 kDa, respectively, decreased the urease activity of *H. pylori* more than the control group (Lim, 2015).

6.3.4 Bacterial vaginosis

Bacterial vaginosis is a vaginal infection caused by an anaerobic bacteria called *Gardnerella vaginalis*. When bacterial vaginosis is not treated precisely, it can cause pelvic infections, infertility, and complications during pregnancy and after pregnancy. In the treatment of bacterial vaginosis, metronidazole, clindamycin, or ornidazole are used as the local and oral antibiotics. These antibiotics cause a high rate of recurrence of the treatment within a month, as they can inhibit natural vaginal microflora with pathogens. Therefore bacteriocins are considered a new treatment option. While Lactocin 160 produced by *Lactobacilli* isolated from vagina of healthy women inhibits vaginal pathogens such as *G. vaginalis* and *Prevotella bivia*, it has not been observed to have any negative effects on healthy flora. Turovskiy, Ludescher, Aroutcheva, Faro, and Chikindas (2009) found that Lactocin 160 targets the cytoplasmic membrane of *G. vaginalis*, alters ATP efflux, and dissipates proton motive force (Turovskiy et al., 2009). Kaur et al. (2013a) examined the effects of approximately 100 bacteriocins produced by LAB strains isolated from vaginal swabs of healthy and fertile women, against *G. vaginalis* and other vaginosis-related bacteria. Since bacteriocins produced by *L. fermentum* HV6b show significant inhibitory activity against *G. vaginalis*, it has been suggested that it can be used in developing a topical formulation for the prevention and treatment of bacterial vaginosis (Kaur et al., 2013a).

6.3.5 Spermicidal effect

Silkin, Hamza, Kaufman, Cobb, and Vederas (2008) found that both bacteriocins exert a spermicidal effect on horse and rat sperms in studies in which they examined the spermicidal activity of Lacticin 3147 and Subtilosin A. On the other hand, the effects of Fermenticin HV6b on diluted semen samples were evaluated on sperm mobility and aggregation, and it was suggested that this bacteriocin can be used as a potent spermicidal agent. Reports have been published in previous studies that nisin, subtilosin, and pediocin CP2 can also be used as a spermicidal agent (Reddy, Aranha, Gupta, & Yedery, 2004; Sutyak et al., 2008; Kumar, Balgir, Kaur, Mittu, & Garg, 2012).

6.3.6 Urinary tract infections

Urinary tract infection refers to bacterial growth in the urinary system and the inflammatory condition caused by this condition. Urinary tract infections show acute, chronic, or recurrent features. Bacteria, fungi, and viruses can cause infections. Antibiotics that can be used in the treatment of urinary system infections are various, but due to the increased resistance rates, new alternative agents should be discovered. For this purpose, studies evaluating the effectiveness of bacteriocins on urinary tract infections have been conducted. Trivedi, Kumar Jena, Kumar Patel, and Seshadri (2013) demonstrated that bacteriocin DT24 produced by *L. brevis* DT24 isolated from vaginal samples of healthy women is effective in the inhibition of various uropathogenic bacteria such as *E. coli*, *E. fecium*, *E. fecalis*, and *Staphylococcus aureus*.

Antimicrobial effects of bacteriocins produced by *Lactobacillus acidophilus* JCM1132, *Lactobacillus fermentam* BCS25, *Lactobacillus plantarum* ATCC8041, and *Lactobacillus casei* XM2-1 were investigated against *E. coli*, *Bacillus subtilis*, *Streptococcus*, *Staphylococcus*, and *Pseudomonas* sp. isolated from the urine of diabetic patients with suspected urinary tract infection. Especially bacteriocins of *L. acidophilus* JCM1132 and *L. fermentam* BCS25 exerted a broad-spectrum antimicrobial effect against all uropathogens tested (Ramasamy & Suyambulingam, 2015).

7 Conclusion

Bacteriocins are one of many substances synthesized by microorganisms and are compounds in the structure of peptides or proteins that are ribosomally synthesized by Gram-positive and Gram-negative bacteria. In this chapter, bacteriocins synthesized by Gram-negative microorganisms were classified as pyocins, klebicins, microsins, colicins, colicin-like, and

tailocins. However, bacteriocins synthesized by Gram-positive microorganisms were grouped as classes I, II, and III. Today, their classification as structural has not been completed. This classification system is being revised day by day with new scientific studies. Due to the resistance to antibiotics and their adverse effects, the beneficial effects of bacteriocins are currently being evaluated in the food industry, human, and animal health. Studies to date have shown that bacteriocins have antibacterial mechanisms of action such as inhibition of cell wall biosynthesis, inhibition of DNA replication and transcription, formation of septum, inhibition of protein synthesis, and membrane disturbance.

They have been found to have important advantages, especially as a food preservative. Nisin from *L. lactis* subsp. *lactis*, acidophilin, lactocidin, and lactocin, from *L. acidophilus*, reuterine from *Lactobacillus reuteri* are bacteriocins used as bioprotectors in foods. On the other hand, since conventional antibiotics used in treatment of animal diseases affect milk quality and productivity, experimental studies have shown that bacteriocins such as Lacticin 3147 can be preferred in treatment. Bacteriocins need to be evaluated in terms of their use as novel and potential biological drug raw materials in human health due to their spermicidal, antiulcer, anticancer, antiviral, and antibacterial effects. Continuing research on bacteriocins is of great importance for human health. As long as studies continue in this area, our findings on bacteriocins will increase over time and new species and their effects will be identified.

References

Al Kassaa, I., Hober, D., Hamze, M., Chihib, N. E., & Drider, D. (2014). Antiviral potential of lactic acid bacteria and their bacteriocins. *Probiotics and Antimicrobial Proteins*, *6*, 177–185. doi: 10.1007/s12602-014-9162-6.

Al-Rubiee, R., Al-Mudaffar, S., Hassan, F., & Al-ani, F. (1988). Purification and characterization of Pyocins R from *Pseudomonas aeruginosa*. *Folia Microbiologica*, *38*, 520–524. doi: 10.1007/BF02925780.

Alvarez-Sieiro, P., Montalbán-López, M., Mu, D., & Kuipers, O. P. (2016). Bacteriocins of lactic acid bacteria: Extending the family. *Applied Microbiology and Biotechnology*, *100*, 2939–2951. doi: 10.1007/s00253-016-7343-9.

Anbari, K., Firouzi, M., & Abbaszadeh, S. (2019). Probiotics and gastrointestinal diseases: A promising complementary medicine resource for treatment of gastrointestinal disorders and diseases. *Journal of Pharmacy & Pharmacognosy Research*, *7*(3), 193–199.

Baindara, P., Gautam, A., Raghava, G. P. S., & Korpole, S. (2017). Anticancer properties of a defensin like class IId bacteriocin Laterosporulin10. *Scientific Reports*, *7*, 46541. doi: 10.1038/srep46541.

Baindara, P., Korpole, S., & Grover, V. (2018). Bacteriocins: Perspective for the development of novel anticancer drugs. *Applied Microbiology and Biotechnology*, *102*, 10393–10408. doi: 10.1007/s00253-018-9420-8.

Bemena, L. D., Mohamed, L. A., Fernandes, A. M., & Lee, B. H. (2014). Applications of bacteriocins in food, livestock health and medicine. *International Journal of Current Microbiology and Applied Sciences*, *3*(12), 924–949.

Bordalo Tonucci, L., Olbrich Dos Santos, K., Lucia De, C., Fortes Ferreira, L., Machado Rocha Ribeiro, S., Licursi De Oliveira, L., et al. (2017). Gut microbiota and probiotics: Focus on diabetes mellitus. *Critical Reviews in Food Science and Nutrition*, *57*(11), 2296–2309. doi: 10.1080/10408398.2014.934438.

Cavera, V. L., Arthur, T. D., Kashtanov, D., & Chikindas, M. L. (2015). Bacteriocins and their position in the next wave of conventional antibiotics. *International Journal of Antimicrobial Agents*, *46*, 494–501. doi: 10.1016/j.ijantimicag.2015.07.011.

Cesa-Luna, C., Baez, A., Quintero-Hernández, V., De la Cruz-Enríquez, J., Castañeda-Antonio, M. D., & Muñoz-Rojas, J. (2020). The importance of antimicrobial compounds produced by beneficial bacteria on the biocontrol of phytopathogens. *Acta Biológica Colombiana*, *25*(1), 140–154. doi: 10.15446/abc.v25n1.76867.

Chen, H., & Hoover, D. G. (2003). Bacteriocins and their food applications. *Comprehensive Reviews in Food Science and Food Safety*, *2*, 82–100. doi: 10.1111/j.1541-4337.2003.tb00016.x.

Cintas, L. M., Casaus, M. P., Herranz, C., Nes, I. F., & Hernández, P. E. (2001). Review: Bacteriocins of lactic acid bacteria. *Food Science and Technology International*, *7*(4), 281–305. doi: 10.1106/R8DE-P6HU-CLXP-5RYT.

Cotter, P. D., Hill, C., & Ross, R. P. (2005). Bacteriocins: Developing innate immunity for food. *Nature Reviews Microbiology*, *3*(10), 777–788. doi: 10.1038/nrmicro1273.

Cotter, P., Ross, R., & Hill, C. (2013). Bacteriocins—A viable alternative to antibiotics? *Nature Reviews Microbiology*, *11*, 95–105. doi: 10.1038/nrmicro2937.

Cornut, G., Fortin, C., & Soulières, D. (2008). Antineoplastic properties of bacteriocins: Revisiting potential active agents. *American Journal of Clinical Oncology*, *31*(4), 399–404. doi: 10.1097/COC.0b013e31815e456d.

Çayır Üstündağ, H., & Yalçın, H. (2017). Bakteriyosinler ve Gıdalarda Kullanımı. *Mehmet Akif Ersoy Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi*, *5*(1), 53–65. doi: 10.24998/maeusabed.299346.

Dever, L. A., & Dermody, T. S. (1991). Bacteriocins: Classification, synthesis, mechanism of action and resistance development in food spoilage causing bacteria. *Microbial Pathogenesis*, *128*, 171–177. doi: 10.1016/j.micpath.2019.01.002.

Dinçer, E., Kıvanç, M., & Karaca, H. (2010). Biyokoruyucu olarak laktik asit bakterileri. *GIDA*, *35*(1), 1–8.

Dorosky, R. J., Yu, J. M., Pierson, L. S., III, & Pierson, E. A. (2017). *Pseudomonas chlororaphis* produces two distinct R-tailocins that contribute to bacterial competition in biofilms and on roots. *Applied and Environmental Microbiology*, *83*, e00706–e00717. doi: 10.1128/AEM.00706-17.

Duport, C., Baysse, C., & Michel-Briand, Y. (1995). Molecular characterization of Pyocin S3, a novel S-type Pyocin from *Pseudomonas aeruginosa*. *The Journal of Biological Chemistry*, *270*, 8920–8927. doi: 10.1074/jbc.270.15.8920.

Ecoy, G., Chaotham, C., Suksomtip, M., Chanchavarote, P., & Arunmanee, W. (2018). Recombinant bacterial-protein Colicin N causes cytotoxicity and oxidative stress in human lung cancer cells. In *The 6th international conference on biochemistry and molecular biology (BMB2018)*Thailand.

Endeshaw, A., Gizaw, B., Tsegay, Z., Tefera, G., & Aynalem, E. (2018). Health benefits of probiotics. *The Journal of Infectious Diseases*, *2*(1), 8–27.

Faustino Jozala, A., De Lencastre Novaes, L. C., & Pessoa Junior, A. (2015). Chapter 5: Nisin. In A. Jozala (Ed.), *Fermentation process* (pp. 103–119.). doi: 10.5772/60932.

Férir, G., Petrova, M. I., Andrei, G., Huskens, D., Hoorelbeke, B., Snoeck, R., et al. (2013). The lantibiotic peptide labyrinthopeptin A1 demonstrates broad anti-HIV and anti-HSV activity with potential for microbicidal applications. *PLoS One*, *8*(5), e64010. doi: 10.1371/journal.pone.0064010.

Fijan, S. (2014). Microorganisms with claimed probiotic properties: An overview of recent literature. *International Journal of Environmental Research and Public Health*, *11*, 4745–4767. doi: 10.3390/ijerph110504745.

Fischer, S., Godino, A., Quesada, J. M., Cordero, P., Jofré, E., Mori, G., et al. (2012). Characterization of a phage-like pyocin from the plant growth-promoting rhizobacterium *Pseudomonas fluorescens* SF4c. *Microbiology*, *158*, 1493–1503. doi: 10.1099/mic.0.056002-0.

Ghazaryan, A. (2013). *The role of stress in bacteriocins regulation*. Israel: Ben Gurion University of the Negev. Doctoral thesis.

Gomes, B. C., Rodrigues, M. R., Winkelstroter, L. K., Nomizo, A., & De Martinis, E. C. P. (2012). In vitro evaluation of the probiotic potential of bacteriocin producer *Lactobacillus sakei* 1. *Journal of Food Protection*, *75*(6), 1083–1089. doi: 10.4315/0362-028X.JFP-11-523.

Gupta, R., Jeevaratnam, K., & Fatima, A. (2018). Lactic acid bacteria: Probiotic characteristic, selection criteria, and its role in human health (a review). *International Journal of Emerging Technologies and Innovative Research*, *5*(10), 411–424.

Güllüce, M., Karadayı, M., & Barış, Ö. (2013). Bacteriocins: Promising natural antimicrobials. *Microbial pathogens and strategies for combating them*. Madrid: Formatex Research Center Press. pp. 1016–1027.

Hassan, M., Kjos, M., Nes, I. F., Diep, D. B., & Lotfipour, F. (2012). Natural antimicrobial peptides from bacteria: Characteristics and potential applications to fight against antibiotic resistance. *Journal of Applied Microbiology*, *113*, 723–736. doi: 10.1111/j.1365-2672.2012.05338.x.

Indira, M., Venkateswarulu, T. C., Peele, K. A., Bobby, N., & Krupanidhi, S. (2019). Bioactive molecules of probiotic bacteria and their mechanism of action: A review. *Biotech*, *9*(306), 2–11. doi: 10.1007/s13205-019-1841-2.

Jacob, F. (1954). Biosynthese induit ot mede d'action d'une pyocine antibiotique de *Pseudomonas pyocyanea*. *Annales de l'Institut Pasteur*, *86*, 149–160.

Kareem, A. A., & Al-Salmani, T. S. (2017). Detection of bacteriocin gene from *Klebsiella Pneumoniae*. *World Journal of Pharmaceutical Research*, *6*(6), 122–128. doi: 10.20959/wjpr20176-8229.

Karpinski, T. M., & Adamczak, A. (2018). Anticancer activity of bacterial proteins and peptides. *Pharmaceutics*, *10*, 54. doi: 10.3390/pharmaceutics10020054.

Kaur, B., Balgir, P. B., Mittu, B., Chuahan, A., Kumar, B., & Garg, N. (2013a). Antimicrobial spectrum of anti-*Gardnerella vaginalis* bacteriocin producing *Lactobacillus fermentum* HV6b against bacterial vaginosis associated organisms. *American Journal of Drug Discovery and Development*, *3*(1), 1–12. doi: 10.3923/ajdd.2013.1.12.

Kaur, B., Balgir, P. B., Mittu, B., Kumar, B., & Garg, N. (2013b). Biomedical applications of fermenticin HV6b isolated from *Lactobacillus fermentum* HV6b MTCC10770. *BioMed Research International*, *2013*, 8. doi: 10.1155/2013/168438. Article ID 168438.

Kaur, B., & Garg, N. (2013). Characteristics of Bacteriocin BA28 produced by *Pediococcus acidilactici* BA28. *Mintage Journal of Pharmaceutical and Medical Sciences*, *2*(1), 18–20.

Kaur, S., & Kaur, S. (2015). Bacteriocins as potential anticancer agents. *Frontiers in Pharmacology*, *6*, 272. doi: 10.3389/fphar.2015.00272.

Kim, T. S., Hur, J. W., Yu, M. A., Cheigh, C. I., Kim, K. N., Hwang, J. K., et al. (2003). Antagonism of *Helicobacter pylori* by bacteriocins of lactic acid bacteria. *Journal of Food Protection*, *66*(1), 3–12. doi: 10.4315/0362-028X-66.1.3.

Kjos, M., Nes, I. F., & Diep, D. B. (2011). Mechanisms of resistance to bacteriocins targeting the mannose phosphotransferase system. *Applied and Environmental Microbiology*, *77*(10), 3335–3342. doi: 10.1128/AEM.02602-10.

Klaenhammer, T. R. (1993). Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiology Reviews*, *12*, 39–86. doi: 10.1111/j.1574-6976.1993.tb00012.x.

Kodali, V. P., Lingala, A. P., Karlapudi, M. I., Venkateswarulu, T. C., & Dulla, J. B. (2013). Biosynthesis and potential applications of bacteriocins. *Journal of Pure and Applied Microbiology*, *7*, 4.

Kumar, B., Balgir, P. P., Kaur, B., Mittu, B., & Garg, N. (2012). Antimicrobial and spermicidal activity of native and recombinant pediocin CP2: A comparative evaluation. *Archives of Clinical and Microbiology*, *3*(3), 1–12. doi: 10.3823/254.

Kumariya, R., Garsa, A. K., Rajput, Y. S., Sood, S. K., Akhtar, N., & Patel, S. (2019). Bacteriocins: Classification, synthesis, mechanism of action and resistance development in food spoilage causing bacteria. *Microbial Pathogenesis*, *28*, 171–177. doi: 10.1016/j.micpath.2019.01.002.

Kurt, Ş, & Zorba, Ö. (2005). Bakteriyosinler ve gıdalarda kullanım olanakları. *Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi*, *16*(1), 77–83. doi: 10.24998/maeusabed.299346.

Lagha, A. B., Haas, B., Gottschalk, M., & Grenier, D. (2017). Antimicrobial potential of bacteriocins in poultry and swine production. *Veterinary Research*, *48*, 22. doi: 10.1186/s13567-017-0425-6.

Lim, E. S. (2015). Purification and characterization of two bacteriocins from *Lactobacillus brevis* BK11 and *Enterococcus fecalis* BK61 showing anti-*Helicobacter pylori* activity. *Journal of the Korean Society for Applied Biological Chemistry*, *58*, 703–714. doi: 10.1007/s13765-015-0094-y.

Linares, D. M., Gómez, C., Renes, E., Fresno, J. M., Tornadijo, M. E., Ross, R., et al. (2017). Lactic acid bacteria and bifidobacteria with potential to design natural biofunctional health-promoting dairy foods. *Frontiers in Microbiology*, *8*, 846. doi: 10.3389/fmicb.2017.00846.

Maryam, H., Maqsood, S., & Farooq, U. (2017). Bacteriocins: A novel weapon against emerging resistance. *Saudi Journal of Pathology and Microbiology*, *2*(7), 220–227. doi: 10.21276/sjpm.

Maqueda, M., Sanchez-Hidalgo, M., Fernández, M., Montalban-Lopez, M., Valdivia, E., & Martinez-Bueno, M. (2008). Genetic features of circular bacteriocins produced by Gram-positive bacteria. *FEMS Microbiology Reviews*, *32*, 2–22. doi: 10.1111/j.1574-6976.2007.00087.x.

Meade, E., Slattery, M. A., & Garvey, M. (2020). Bacteriocins, potent antimicrobial peptides and the fight against multi drug resistant species: Resistance is futile? *Antibiotics*, *9*(1), 32. doi: 10.3390/antibiotics9010032.

Michel-Briand, Y., & Baysse, C. (2002). The pyocins of *Pseudomonas aeruginosa*. *Biochimie*, *84*, 499–510. doi: 10.1016/s0300-9084(02)01422-0.

Osama, O. I. (2019). Classification of antimicrobial peptides bacteriocins, and the nature of some bacteriocins with potential applications in food safety and bio-pharmaceuticals. *EC Microbiology*, *15*(7), 591–608.

Oscáriz, J., & Antonio, P. (2001). Classification and mode of action of membrane-active bacteriocins produced by Gram-positive bacteria. *International Microbiology*, *4*, 13–19. doi: 10.1007/s101230100003.

Parada, J. L., Ricoy, C. C., Medeiros, A. B. P., & Soccol, C. R. (2007). Bacteriocins from lactic acid bacteria: Purification, properties and use as biopreservatives. *Brazilian Archives of Biology and Technology*, *50*(3), 512–542. doi: 10.1590/S1516-89132007000300018.

Punyauppapath, S., Phumkhachorn, P., & Rattanachaikunsopon, P. (2015). Nisin: Production and mechanism of antimicrobial action. *International Journal of Current Research and Review*, *7*(2), 47–53. doi: 10.1016/j.ifset.2018.06.008.

Quintana, V. M., Torres, N. I., Wachsman, M. B., Sinko, P. J., Castilla, V., & Chikindas, M. (2014). Antiherpes simplex virus type 2 activity of the antimicrobial peptide subtilosin. *Journal of Applied Microbiology*, *117*, 1253–1259. doi: 10.1111/jam.12618.

Ramasamy, T. K., & Suyambulingam, K. (2015). Molecular characterization of *Lactobacillus* sp, from Indian curd and its antagonistic effects on uropathogens of diabetic patients. *International Research Journal of Biological Sciences*, *4*(8), 14–22.

Ramu, R., Shirahatti, P. S., Devi, A. T., Prasad, A., Kumuda, J., Lochana, M. S., et al. (2015). Bacteriocins and their applications in food preservation. *Critical Reviews in Food Science and Nutrition*, *2015*doi: 10.1080/10408398.2015.1020918.

Rebuffat, S. (2012). Microcins in action: Amazing defence strategies of Enterobacteria. *Biochemical Society Transactions*, *40*(6), 1456–1462. doi: 10.1042/BST20120183.

Reddy, K. V. R., Aranha, C., Gupta, S. M., & Yedery, R. D. (2004). Evaluation of antimicrobial peptide nisin as a safe vaginal contraceptive agent in rabbits: In vitro and in vivo studies. *Reproduction*, *128*(1), 117–126. doi: 10.1530/rep.1.00028.

Ryan, M. P., Meaney, W. J., Ross, R. P., & Hill, C. (1998). Evaluation of Lacticin 3147 and a teat seal containing this bacteriocin for inhibition of mastitis pathogens. *Applied and Environmental Microbiology*, *64*(6), 2287–2290.

Silkin, L., Hamza, S., Kaufman, S., Cobb, S. L., & Vederas, J. C. (2008). Spermicidal bacteriocins: Lacticin 3147 and Subtilosin A. *Bioorganic & Medicinal Chemistry Letters*, *18*(10), 3103–3106. doi: 10.1016/j.bmcl.2007.11.024.

Simons, A., Alhanout, K., & Duval, R. E. (2020). Bacteriocins, antimicrobial peptides from bacterial origin: Overview of their biology and their impact against multidrug-resistant bacteria. *Microorganisms*, *8*, 639. doi: 10.3390/microorganisms8050639.

Suskovic, J., Kos, B., Beganovic, J., Lebos Pavunc, A., Habjanic, K., & Matosic, S. (2010). Antimicrobial activity—The most important property of probiotic and starter lactic acid bacteria. *Food Technology and Biotechnology*, *48*(3), 296–307.

Sutyak, K. E., Anderson, R. A., Dover, S. E., Feathergill, K. A., Aroutcheva, A. A., Faro, S., et al. (2008). Spermicidal activity of the safe natural antimicrobial peptide subtilosin. *Infectious Diseases in Obstetrics and Gynecology*, *2008*, 6 pages. doi: 10.1155/2008/540758. Article ID 540758.

Swe, P. M., Cook, G. M., Tagg, J. R., & Jack, R. W. (2009). Mode of action of dysgalacticin: A large heat-labile bacteriocin. *Journal of Antimicrobial Chemotherapy*, *63*, 679–686. doi: 10.1093/jac/dkn552.

Tagg, J. R., Dajani, A. S., & Wannamaker, L. W. (1976). Bacteriocins of Gram-positive bacteria. *Bacteriological Reviews*, *40*(3), 722–756.

Tymoszewska, A., Diep, D. B., Wirtek, P., & Aleksandrzak Piekarczyk, T. (2017). The non-lantibiotic bacteriocin Garvicin Q targets Man-PTS in a broad spectrum of sensitive bacterial genera. *Scientific Reports*, *7*(1), 8359. doi: 10.1038/s41598-017-09102-7.

Todorov, M., Botes, C., Guigas, U., Schillinger, I., Wiid, M. B., Wachsman, W. H., et al. (2008). Boza, a natural source of probiotic lactic acid bacteria. *Journal of Applied Microbiology*, *104*, 465–477. doi: 10.1111/j.1365-2672.2007.03558.x.

Trivedi, D., Kumar Jena, P., Kumar Patel, J., & Seshadri, S. (2013). Partial purification and characterization of a Bacteriocin DT24 produced by probiotic vaginal *Lactobacillus brevis* DT24 and determination of its anti-uropathogenic *Escherichia coli* potential. *Probiotics Antimicrobial Proteins*, *5*(2), 142–151. doi: 10.1007/s12602-013-9132-4.

Turovskiy, Y., Ludescher, R. D., Aroutcheva, A. A., Faro, S., & Chikindas, M. L. (2009). Lactocin 160, a bacteriocin produced by vaginal *Lactobacillus rhamnosus*, targets cytoplasmic membranes of the vaginal pathogen, *Gardnerella vaginalis*. *Probiotics Antimicrobial Proteins*, *1*(1), 67–74. doi: 10.1007/s12602-008-9003-6.

Uchechukwu, U. N., Green, E., & Okoh, A. I. (2012). Bacterial exopolysaccharides: Functionality and prospects. *International Journal of Molecular Sciences*, *13*, 14002–14015. doi: 10.3390/ijms131114002.

Vijayaram, S., & Kannan, S. (2018). Probiotics: The marvelous factor and health benefits. *Biomedical and Biotechnology Research Journal*, *2*, 1–8.

Wang, Z. B., Xin, S. S., Ding, L. N., Ding, W. Y., Hou, Y. L., Liu, C. Q., et al. (2019). The potential role of probiotics in controlling overweight/obesity and associated metabolic parameters in adults: A systematic review and meta-analysis. *Evidence-Based Complementary and Alternative Medicine*, *2019*, 14. doi: 10.1155/2019/3862971. Article ID 3862971.

World Gastroenterology Organisation Global Guidelines. (2017). *Probiotics and prebiotics*. Croatia: InTechOpen. https://www.worldgastroenterology.org/guidelines/global-guidelines/probiotics-and-prebiotics/probiotics-and-prebiotics-english).

Yang, S. C., Lin, C. H., Sung, C. T., & Fang, J. Y. (2014). Antibacterial activities of bacteriocins: Application in foods and pharmaceuticals. *Frontiers in Microbiology*, *5*, 241. doi: 10.3389/fmicb.2014.00241.

Yangılar, F. (2015). Probiyotik Mikroorganizmaların Biyokoruyucu Özelliği. *Uludağ Üniversitesi Mühendislik Fakültesi Dergisi*, *20*(1), 119.

Zainodini, N., Hassanshahi, G., Hajizadeh, M., Khanamani Falahati-Pour, S., Mahmoodi, M., & Mirzaei, M. R. (2018). Nisin induces cytotoxicity and apoptosis in human asterocytoma cell line (SW1088). *Asian Pacific Journal of Cancer Prevention*, *19*(8), 2217–2222. doi: 10.22034/APJCP.2018.19.8.2217.

Liu, Wenjun, Pnag, Huili, Zhang, Heping, & Cai, Yimin (2014). Biodiversity of Lactic Acid Bacteria. *Lactic Acid Bacteria*. Springer.

Kortel, R., & Moreno, F. (1992). Genetics of ribosomally synthesized peptide antibiotics. *Annual Review of Microbiology*, *46*, 141–163.

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Chapter 19

Probioactives: Bacteriocin and Exopolysaccharides

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1 Introduction

Probiotics are live microorganisms and, when administered in adequate amounts, confer health benefits on the host (Hill et al., 2014; Gibson et al., 2017). Most probiotic bacteria are lactic acid bacteria (LAB) that include a ubiquitous and heterogeneous species with common feature of lactic acid production as the majority end product. Taxonomically, LAB species include two distinct phyla, namely, Firmicutes and Actinobacteria (Carr et al., 2002; Argyri et al., 2013). In Firmicutes phylum, LAB belong to the *Lactobacillales* order and include *Lactobacillus* spp., *Lactococcus* spp., *Pediococcus* spp., *Leuconostoc* spp., *Streptococcus* spp., *Oenococcus* spp., *Enterococcus* spp., *Aerococcus* spp., *Tetragenococcus* spp., *Carnobacterium* spp., *Weissella* spp., *Alloiococcus* spp., *Vagococcus* spp., and *Symbiobacterium* spp. (Reddy et al., 2008). Despite *Lactobacillus* species, the term probiotic can be extended to other microorganisms including *Bacillus* spp., *Saccharomyces* spp., *Propionibacteria* spp., and *Sporolactobacillus* spp., (De Vrese & Schrezenmeir, 2008).

A number of therapeutic applications of probiotics have been reported, including chronic diseases such as hypercholesterolemia, food allergy, control of irritable bowel syndrome, inflammatory bowel diseases, and symptoms of the HIV-infected individual (Gorska et al., 2019). The mode of action of probiotics can be classified as follows:

1. Modulation of host's defenses including the innate and acquired immune system, such as treating the gastrointestinal tract (GIT) inflammation, prevention and therapy of infectious diseases.
2. Direct effect on commensal and/or pathogenic microorganisms;
3. Microbial products like toxins and host products, for example, bile salts and food ingredients (Rossi et al., 2018)

Over the last few years, the consideration that viability of probiotics is not a crucial characteristic to exert its beneficial effects has increased the attention (Lebeer et al., 2010). More recently, the researchers have shifted their focus to identify the novel bacteria-derived defined molecules, so-called probioactives. These molecules may represent safer alternative to chemical preservatives, antibiotic growth promoters (AGP), and food stabilizers while retaining the desired effects on the host or food products. This chapter explores an overview of the probioactives concept, evidence of their beneficial effects as well as their mode of action involved in their protective effects and current status on the potential applications of probioactive molecules [bacteriocin and exopolysaccharide (EPS)] in foods and pharmaceutical industries.

2 Probioactive perception

Human and animal body possesses various microbes in their gut environment, especially the bacteria, fungi, and viruses (Cani, 2018). The growth of this gut microflora is triggered by different factors such as diet, human health, infections, life style, aging, and host immunity; therefore, the gut environment is changed (Conlon & Bird, 2014). The human gut microbiome is a promising source for novel therapeutics in probiotic form to produce bioactive molecules that address the

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00019-8
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health issues like microbial infections, obesity, metabolic disorders, and host immunity (Cani, 2018; Indira et al., 2019) Hence, many bioactive compounds have now been identified from probiotic strains and classified them under probioactive molecules/compounds (Champagne et al., 2018).

The term "probioactive" represents the bioactive compound, which is directly linked to the presence or activity of probiotic bacteria (Farnworth & Champagne, 2010; Champagne et al., 2018). These probioactive molecules are vitally crucial for healthy lifestyle, which stimulates the beneficial effects on both human and animal through numerous mechanisms (Chong, 2014; Rahbar Saadat et al., 2019). Currently, these molecules are one of the most promising compounds to be used as food preservatives due to their broad spectrum of inhibition against a variety of Gram-positive bacteria and modulate the host's natural defense mechanisms (Deepak et al., 2016; Zannini et al., 2016). Probioactive molecules secreted by probiotic microorganism including LAB have been considered to be "Generally regarded as safe molecules" that provide health impacts to host (Baindara et al., 2018).

3 Sources and strain specificity of probioactives

The probioactives can be identified from two different sources. The first form of source is direct synthesis of the probioactives by the probiotic strains. Bacteriocins, EPSs, or enzymes come under this category, which are present in either supplements or foods. The second form of source is a compound that is formed by the modification of the food matrix itself by the probiotic strain. One of the suitable examples for this category is peptides specifically released from hydrolysis of milk casein. These probioactives are only present in fermented foods and are directly linked to nature of the food matrix (Leclerc et al., 2002).

Variations between probiotic species and strains are considered to be one of the prerequisites with respect to the synthesis of probioactives. The synthesis of bacteriocin and EPS varies strongly between species (Tanaka et al., 1999) as well as within strains in a given species (Corzo & Gilliland, 1999). Hence, we cannot conclude that all probiotic strains, or strains of a given species, could produce a probioactive at same levels. Therefore, the strain used to synthesize/produce a probioactive is crucial to its functionality. As a first step, the functionality of a probiotic strain can be assured by its characterization study (Champagne et al., 2018). Regarding this, many researches should be undertaken to characterize the current probiotic strains showing health benefits, which have the ability to produce probioactives like bacteriocin (biopreservative, AGP) and EPS (texture, adsorption of toxic substances such as heavy metals). Thus, the scope and importance of those probiotic strains producing probioactive molecules like bacteriocin and EPS will become enlarged in future.

4 Bacteriocin from probiotic strains

Probiotic microorganisms have been used in food and feed preservatives for many centuries as they can produce several antimicrobial agents, including organic acids (lactic and acetic acid), ethanol, carbon dioxide, diacetyl, hydrogen peroxide, and bacteriocins (Vieco-Saiz et al., 2019). Among them, bacteriocins gain more attention due to their broadest antimicrobial spectrum against various foodborne pathogens. Bacteriocins are ribosomally synthesized antimicrobial peptides (AMPs) that have a bactericidal or bacteriostatic effect on other bacterial or fungal species (Cotter et al., 2013). Bacteriocin production is considered as a beneficial characteristic of a probiotic microorganism as they find potential therapeutic applications in host protection against various enteric pathogens, including multiple drug resistant bacteria (Lavilla-Lerma et al., 2013). Among the Gram-positive organisms, LAB are the well-known bacteriocin producers (Jackson et al., 2019; Sanders et al., 2018). Hence, bacteriocins from LAB have a great potential as natural food preservatives that could replace chemical preservatives (Wang et al., 2014). Bacteriocins produced by several LAB have high antilisterial activity, which has driven interest for its overproduction by genera *Bacillus* spp., *Lactococcus* spp., *Enterococcus* spp., and *Pediococcus* spp. Hence, the biotechnological interest would be to design and construct the recombinant probiotic strains for constitutive or controlled heterologous production of bacteriocins with maximum antimicrobial activity against *Listeria* spp. (Cotter et al., 2013; Jimenez et al., 2015).

Moreover, the antagonistic effect of bacteriocins is a successful strategy for maintaining population, reducing numbers of competitors and pathogens to obtain more nutrients and living space in GIT (Yang et al., 2014). This kind of defensive characteristic helps probiotic strain to occupy a specific niche and also restricts the transmission of pathogens to neighboring cells. Recent studies reported that probiotic strains are mediating quorum-sensing signals in bacterial biofilms (Mukherjee & Ramesh, 2015; Dobson et al., 2012; Indira et al., 2019; Kareb & Aider, 2020). Furthermore, the high percentage of hydrophobicity by the peptide bacteriocin could exert cytotoxic effect. This promotes interaction of probiotic microorganisms with the host cell membranes, thereby inhibiting the cancer cell proliferation (Górska et al., 2019). However, it should possess nontoxic effect on normal cells to exploit the bacteriocin as a food, feed additive, and biopreservative (Das & Goyal, 2014).

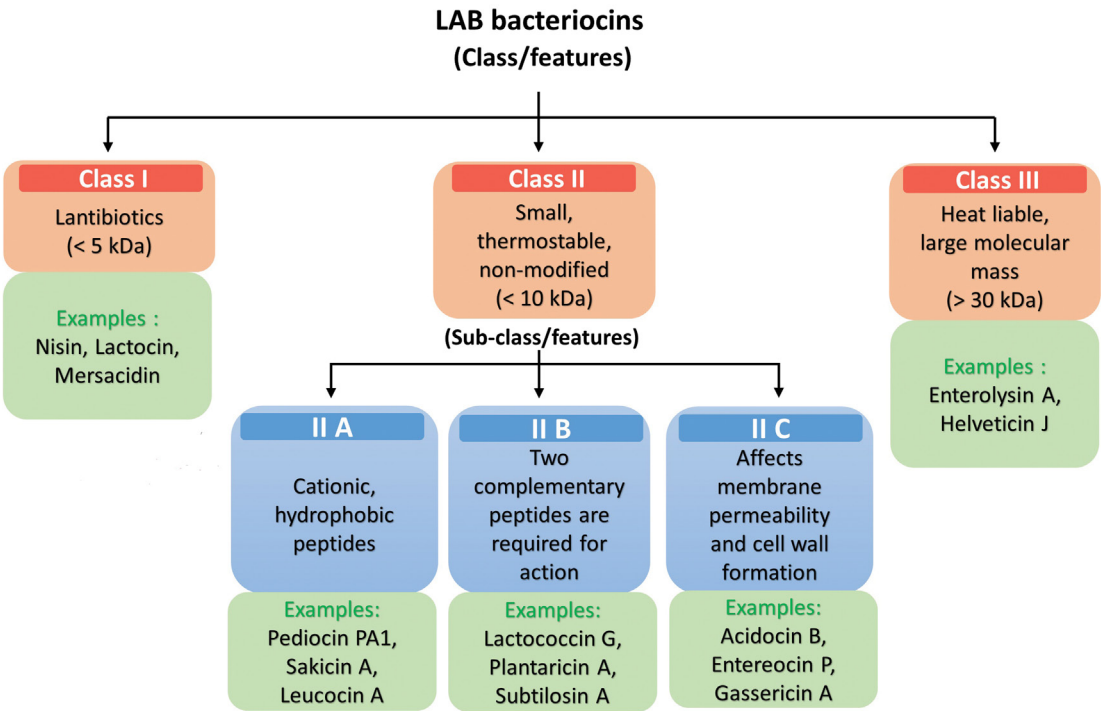


FIGURE 19.1 Classification of bacteriocin.

5 Bacteriocin classification

Bacteriocin varies in their molecular weight, biochemical characteristics, spectrum, and mode of their action. These unique and different characteristics have made their classification quite complex and controversial (Zouhir et al., 2010; Kareb & Aider, 2020; O'Connor et al., 2020). Bacteriocins produced by Gram-positive are classified into different classes based on their structural and functional characteristics presented in Fig. 19.1. The class I bacteriocins are small (<5 kDa), heat-stable, and highly posttranslationally modified peptides containing characteristic polycyclic thioether amino acids like nisin and lacticin. Class II bacteriocins are small (<10 kDa), heat stable, and include non-posttranslationally modified proteins like subtilosin A (sboA), gassericin A, circularin A, and carnocyclin A. Class III bacteriocins are high-molecular-weight (>30 kDa) and heat-labile proteins like colicins, klebicin megacins, and enterolysin (Cotter et al., 2013).

6 Biochemical characterization of bacteriocin

Physicochemical properties of bacteriocins like stability at acidic and alkaline pH, heat, organic solvent, and proteases show great potential to be used as food biopreservatives instead of chemical preservatives. LAB bacteriocins are sensitive to digestive proteases like trypsin, pancreatin complex, and chymotrypsin and hence do not negatively impact the gut microbiota (Egan et al., 2016). Many of the LAB bacteriocins are only stable at neutral and acidic pH and are inactivated above pH 8.0 (e.g., nisin and pediocin AcH). They possess heat stability ranging from 60°C or 100°C for about 30 min and to autoclaving at 121°C for 15–20 min (e.g., lactacin B and lactacin F) (De Vuyst & Vandamme, 1994). Sutyak et al., (2008) reported that most of the bacteriocins produced by LAB are stable at acid and neutral pH and are inactivated at a pH above 8.0 (nisin, leucocin A-UAL 187, and pediocin AcH) and stable at varying temperatures ranging from 60°C or 100°C for more than 30 min (sboA, lactocin S, lactocin 27, and carnobacteriocins A and B) to autoclaving at 121°C for 15–20 min (lactacin B, nisin, and lactacin F). Gassericin S (GS) produced by Lactobacillus gasseri LA327 (human large intestine isolate) was stable in a wide pH range (pH 2, 4, 7, and 10), and 25% of GS activity was retained under the extreme heating condition (121°C, 15 min). The authors reported that GS completely lost activity upon treatment with proteases used in this study (Kasuga et al., 2019).

7 Bacteriocin genetics and biosynthesis

Primarily, bacteriocins are synthesized as precursor or prepeptides that are biologically inactive and contain an N-terminal extension or leader sequence. Subsequence cleavage of this prepeptide at a specific processing site removes the leader sequence from the antimicrobial molecule concomitantly with its export to the outside of the cell. This translocation of

mature bacteriocin across the cytoplasmic membrane is mediated by ATP-binding cassette (ABC)-transporter and accessory protein (Khochamit et al., 2015).

Generally, the genes encoding bacteriocin production and responsible for immunity are arranged in operon clusters. They can also be located on mobile genetic elements like in chromosome associated with plasmids or transposons. Newly synthesized bacteriocins contain an N-terminal leader, which are modified by proteins or amino acids encoded by the bacteriocin gene cluster before they are exported out of the cell. The production of bacteriocin requires four types of genes, namely, bacteriocin structural genes (encodes the bacteriocin precursor), an immunity gene (protects the producer from its own bacteriocin), a membrane-associated ABC transporter, and its accessory protein. Fluhe et al. (2012) postulated the biosynthetic pathway for sboA maturation, namely, (1) ribosomal assembly of sboA; (2) formation of thioether bond catalyzed by AlbA, introducing three intramolecular bridges and delivering N- and C-terminal amino acids of the core peptide in nearby proximity; (3) proteolytic cleavage of leader peptide catalyzed by putative proteases AlbE, AlbF, or both and macrocylization of 35 amino acid residue triply bridged core peptide; and (4) transport of matured sboA by ABC-type export protein AlbC. Synthesized bacteriocins were exported by the ABC transporter that is carrying a proteolytic domain responsible for leader sequence cleavage after the double-glycine residues in bacteriocins (Birri et al., 2010).

Bacteriocin-producing probiotic strains protect themselves from their own bacteriocin by producing specific immunity peptides. Genes encoding these proteins are placed in close genetic proximity to several other types of structural and processing genes of bacteriocin. The immunity and the structural bacteriocin genes are located on the same operon. Lantibiotics are encoded by structural gene(s) that are referred as lanA (lan refers to homologous genes of various lantibiotic gene clusters). The genes required for modification (lanB, lanC, lanM, lanD, and lanJ), ABC-type peptide translocators (lanT), proteolytic processing (lanP), immunity (lanI, FEG), and regulation (lanR, lanK, lanQ, lanX) are usually present in close proximity. Both the Lan I and the Lan EFG (multicomponent ABC transporter) systems have been described for LAB. The Lan I protein, in particular, provides immunity to producer cells by preventing pore formation by their own bacteriocins that have been inserted into the membrane by pushing them back, thereby keeping the bacteriocin concentration in the membrane under control (Todorov, 2009; Zacharof & Lovitt, 2012).

Bacteriocins with maximum antimicrobial activity against bacterial pathogens could be overproduced, which would contribute to the higher probiotic effect of recombinant probiotic strains. Hence, designing and construction of recombinant probiotics strains would lead to the controlled or constitutive heterologous production of bacteriocins with enhanced antimicrobial activity (Jimenez et al., 2015).

8 Cytotoxicity effect of bacteriocin

The safe use of bacteriocins requires the absence of cytotoxicity to human and cells. The pathogenic potential of probiotic microorganism is considered quite low. Very rare cases like endocarditis have been reported. However, further investigation is necessary for probiotic and their metabolites use in human populations and animals such as severely immune-compromised subjects, neonates, or hospitalized patients. Hence, the use of appropriate in vitro assay, that is, cytotoxicity assay should be demonstrated to assess the safety of probiotics (Messaoudi et al., 2012). Bacteriocin from the probiotic strain *Bacillus subtilis* showed >70% cell viability on the mammalian vero cell line. Moreover, scanning electron microscopy depicted that the bacteriocin was able to disrupt the bacterial cell membrane as its possible mode of action (Sharma et al., 2018) Paiva et al. (2012) demonstrated the cytotoxic effect of nisin to MCF-7 (human breast adenocarcinoma cell line) and HepG2. At highest concentration of 140 μM, the cell viabilities of both the cell lines were found to be less than 20%, and they reported that this reduction in cell viability of cancer cell lines was shown to be dose-dependent. In another study, the cytotoxic compound of *Bacillus vallismortis* showed direct cytotoxic and apoptotic effects on HCT 116 colon cancer cells in a dose- and time-dependent manner (Jeong et al., 2008).

9 Bacteriocin from *Lactobacillus* sp.

Bacteriocin from *Lactobacillus* sp. has been used as biopreservatives in different foods either alone or in combination with other preservations methods. Schillinger and Lücke (1989) reported that Lactobacillus *sakei* isolated from fresh meat produced sakacin-A bacteriocin that exhibited antagonistic activity against *Listeria monocytogenes*. Plasmid profile analysis of *L. sakei* and two bacteriocin-negative variants of this strain indicated that a plasmid of about 18 MDa may be involved in the formation of bacteriocin and immunity to this antibacterial compound.

(Hu et al., 2013) purified plantaricin, produced by Lactiplantibacillus *plantarum*. Plantaricin was stable at high temperature (20 min, 121°C), active in the acidic pH (3–5), sensitive to protease, and exhibited broad-spectrum antimicrobial activity against closely related bacteria. Das and Goyal (2014) purified plantaricin from *L. plantarum* with a molecular size of 15.2 kDa. Plantaricin was sensitive to proteolytic enzymes but stable in the pH range of 2–10. It was resistant to 121°C

for 15 min and remained active upon detergents and surfactants. Therdtatha et al. (2016) isolated Lactobacillus *salivarius* from the duck intestine and purified a bacteriocin, salivaricin exhibiting maximum inhibitory activity against Enterococcus *fecalis* and Listeria *innocua*.

10 Gassericin from *Lactobacillus gasseri*

Among various classes of bacteriocins, circular bacteriocins have gained increased attention, since they are the simplest prokaryotic representatives of the universal circular peptides with numerous physiological properties. Their N and C termini are joined by peptide bonds to produce a circular molecule. Gassericin A or Reutericin 6 is the first example of circular protein produced by *L. gasseri* (Kawai et al., 1998). It includes a head-to-tail cyclization, made up of 58 amino acids with isoleucine at the N-terminal and alanine at the C-terminal, and has a molecular weight of 5652 Da (Kawai et al., 2001). This gassericin A is a cationic molecule and is very hydrophobic. It enables the cell membrane permeability to small molecules, so that they disrupt the proton motive force, which finally leads to cell death (Pandey et al., 2013). It exhibits antimicrobial activity against several foodborne pathogenic bacteria, including *L. monocytogenes*, Bacillus *cereus*, and *Staphylococcus aureus* (Kawai et al., 2001). Hence, Gassericin A can be used as a vital protein in the food preservation because of its properties of high pH and temperature tolerance.

Several authors already purified the bacteriocins of *L. gasseri* using various purification methods. (Kawai et al., 1994) purified gassericin A from *L. gasseri* isolated from feces of human infant. In another study, Kawai et al. (2000) reported that gassericin T produced by *L. gasseri* from human feces showed inhibitory effect on foodborne pathogens, namely, *L. monocytogenes* and *S. aureus*. (Tahara et al., 1997) purified four types of gassericin (B1–B4) from the culture broth of *L. gasseri*. Based on the amino acid sequencing, they found that the only difference between gassericin B1 and B3 is the presence of N-terminal glycine residue in B3. Other than *Lactobacillus* sp., many other LAB would produce gassericin. Maldonado-Barragan et al., (2016) have found the production of gassericin E, a novel bacteriocin produced by *L. gasseri* that was isolated from the vagina, exhibited inhibitory activity against vaginal pathogens. *L. gasseri* used as potential probiotic bacteria to fight and/or prevent bacterial infections as bacterial vaginosis.

11 Bacteriocin from *Bacillus* species

In spite of extensive research on LAB bacteriocins, increasing attention has been addressed to the AMPs-produced *Bacillus* sp., because the production of bacteriocin and sporulation capacity confers *Bacillus* strains with a double advantage in terms of their survival in various habitats (Beric et al., 2014). While LAB bacteriocins usually have a narrow spectrum of antimicrobial property on closely related species, bacteriocins from *Bacillus* sp. have more diverse characteristics and broader spectra of inhibition. Previously, *Bacillus* sp. bacteriocins were classified based on the characteristics of those produced by LAB. But as per the increasing study reports of bacteriocins from *Bacillus* sp., separate classification of LAB bacteriocins has been proposed. They are divided into three classes, namely, class I, posttranslationally modified peptides (large proteins: lantibiotics); class II, nonmodified peptides (small linear peptides); and class III, large heat-labile peptides (Abriouel et al., 2011).

Several important groups of bacteriocin produced by *Bacillus* sp. were cerecidins produced by *B. cereus*; coagulin produced by Bacillus *coagulans*; subtilisin, subtilin, sboA produced by *B. subtilis* and bacthuricin, entomocin, thuricin produced by *Bacillus thuringiensis.* Even though they exhibit promising properties as potential antimicrobial agents, only a few bacteriocins from the genus *Bacillus* have been completely characterized. The molecular weights of the bacteriocins produced by *Bacillus* sp. vary in size, ranging from 1.82 to 21 kDa (Abriouel et al., 2011).

Sabate and Audisio (2013) isolated three surfactin-producing *B. subtilis* strains, C4, M1, and G2III from honey and intestines of *Apis mellifera* L. bee. All surfactins have shown antagonistic activity against six different bacteriocin-resistant *L. monocytogenes* strains. Licheniocin was identified and purified from *Bacillus licheniformis* and was effective against *L. monocytogenes*, methicillin-resistant *S. aureus*, and β-hemolytic *Streptococci* (Beric et al., 2014). (Chopra et al., 2014) identified a novel bacteriocin called sonorensin produced by *Bacillus sonorensis*. This bacteriocin exhibited antimicrobial activity against common foodborne pathogens such as *L. monocytogenes* and *S. aureus*.

12 Subtilosin A from *Bacillus subtilis*

B. subtilis has the ability to produce many antimicrobial substances, including the bacteriocin sboA (Stein, 2005; Cherif et al., 2008; Abriouel et al., 2011; Mouloud et al., 2013) (Cherif, Rezgui, Raddadi, Daffonchio, & Boudabous, 2008; Mouloud, Daoud, Bassem, Laribi Atef, & Hani, 2013) sboA was originally isolated from a wild strain *B. subtilis* (Babasaki et al., 1985). Later, Zhang et al., (1999) published a complete amino acid sequence of sboA. Structural elucidation was provided using ^{1}H-NMR and produced a 3D image of the sboA (Marx et al., 2001). It was found to be a circular molecule

of 35 amino acids. It has a unique posttranslational structure, namely, three sulfur cross-links between cysteine, α-carbon of two phenylalanines, and a threonine residue (Kawulka et al., 2004; Liu et al., 2015). (Parveen Rani et al., 2016) demonstrated the antilisterial sboA production by probiotic strain *Bacillus tequilensis*, an isolate of native chicken intestine. N-terminal amino acid sequencing of purified sboA showed 100% consensus to antilisterial peptide sboA and sboA gene, which was amplified from *B. tequilensis* genome. Sutyak et al. (2008) reported that sboA exhibits bactericidal activity against a wide range of Gram-positive and Gram-negative pathogens especially the foodborne pathogen *L. monocytogenes*, clinical isolates of *Streptococcus agalactiae*, and *Gardnerella vaginalis*. Perez et al. (2017) reported presence of genes encoding pre-sboA in *B. subtilis*, which showed antimicrobial activity against *L. monocytogenes*. Torres et al. (2013) isolated sboA from *Bacillus amyloliquefaciens*, and this is the first report to demonstrate antiviral activity. sboA is a highly posttranslationally modified macrocyclic bacteriocin produced by other *B. subtilis* strains (Abriouel et al., 2011).

13 Bacterial exopolysaccharides

Several probiotic microorganisms are capable of synthesizing a wide variety of carbohydrates including storage polymers (glycogens) located into the cytoplasm or structural polymers (glycans) that form a part of microbial envelope. Glycan polymers can be covalently bound to cell surface to form capsular polysaccharide, loosely attached to the extracellular layer, or secreted outside during cell growth as EPS (Schmid et al., 2015; Rani et al., 2017; Poole et al., 2018). These probiotic EPS have received special attention as they play a critical role in the interaction between pathogens and host, which might play an important role on bacterial adhesion, aggregation, and colonization to intestinal cell wall (Dertli et al., 2015). EPS is a high-molecular-weight, extracellular biopolymer that is produced during the metabolic process of microorganisms. Among the large variety of EPS-producing bacteria, LAB have gained more attention because of remarkable property of the polymers produced by them which do not have any health risk. The usage of EPS-producing probiotics could result in a safe, natural, and healthy end product with better texture and stability (Ismail & Nampoothiri, 2010; Shao et al., 2015). Details of some EPS-production sources, composition, properties, and applications are presented in Table 19.1.

Generally, bacterial EPS are protective in nature; thus, the production of EPS may increase the resistance of the probiotic against unfavorable environmental conditions. The bacterial EPS may enhance probiotic colonization by cell–cell interaction in GIT (Kanmani et al., 2011) and exhibits antimicrobial, immunostimulatory, antioxidant, and antitumor activities (Pan & Mei, 2010; Zhang et al., 2013; Ibarburu et al., 2015). Abundance of EPS around the cells greatly influences bacterial aggregation (Dertli et al., 2015; Wang et al., 2015), cell surface hydrophobicity and biofilm formation, thus competitively excludes adhesion of pathogens on intestinal cell lines (Collado et al., 2008; Walter et al., 2008). In food industry, probiotic EPSs are used as natural alternatives to commercial stabilizers because of their stabilizing, viscosifying, emulsifying, or gelling properties for improving the texture, rheology, and mouth-feel of the fermented products (Venkatesh et al., 2016).

14 EPS characteristics

The general properties of EPS are biodegradability, biocompatibility, renewability, flexibility, eco-friendliness and biomedical properties such as antioxidant, anticarcinogenic, antiinflammatory, and hyperglycemic inhibitor (Shao et al., 2015). Furthermore, EPS can be used as bioabsorbants, bioflocculants, encapsulating materials, drug delivery agents, heavy metal removing agents, ion exchange resins, and hosts for hydrophobic molecules. The polysaccharides could protect bacterial cells from desiccation, antibiotic, penetration of toxic metals, phage attack, and phagocytosis. In recent years, bacterial polysaccharides have become an alternative of interest as antitumor, antiinflammatory, immunostimulatory, immunomodulatory, antiviral, and antioxidant agents in several medical and pharmaceutical industries (Ismail & Nampoothiri, 2010; Shao et al., 2015; Trabelsi et al., 2015).

15 EPS classification

Based on their chemical composition, EPS have been divided into two classes: homopolysaccharides (HoPS) and heteropolysaccharides (HePS). HoPS are generally synthesized from sole sucrose substrate by action of sucrase enzyme. Sucrase synthesizes polysaccharides consisting of either glucose (glucan) or fructose (fructan) residues (Trabelsi et al., 2015; Zhou et al., 2016). HePS consist of multiple types of sugar moieties and synthesized by the combined action of different types of glycosyl transferase enzymes.

HoPS consist of a single type of monosaccharide (α-D-glucans, β-D-glucans, fructans, and others represented by polygalactan) and further divided into two important groups based on the features of their primary structure such as the pattern of main chain bonds, molecular weight, and branch structure. They are (1) α-glucans, mainly composed of α-1,6- and

TABLE 19.1 Details of major exopolysaccharides produced by probiotic bacteria.

	Organism	Source	EPS yield	Molecular weight	Polysaccharide composition	Property/ application	References
Lactobacillus spp.	*L. plantarum* WLPL04	Human breast milk	426.73 ± 65.56 mg/L	6.61×10^4 Da	Xylose, glucose and galactose	Antitumor activity	Liu et al. (2017)
	L. paracasei DG	Vaginal isolate	20–25 mg/500 mL		Rhamnose, D-galactose, and *N*-acetyl-D-galactosamine	Immunostimulatory property	Balzaretti et al. (2017)
	L. casei SB27	Yak milk		LW1—25.10 kDa	Galactose, glucose, and arabinose	Antitumor activity	Di et al. (2017)
				LW2—12.34 kDa	Galactose, glucose, and mannose	Antitumor activity	Di et al. (2017)
	L. gasseri FR4	Native chicken intestine	64.32 ± 0.18 mg/mL	1.86×10^5 Da	Glucose, mannose, galctose, rhamnose, and fucose	Antioxidant activity	Rani, Anandharaj, and David Ravindran (2018)
Bifidobacterium spp.	*B. breve* lw01	Infant feces	26 mg/L		Rhamnose, arabinose, galactose, glucose, and mannose	Anticancer activity	Wang et al., (2019)
	B. longum W11	Commercial		2.8×10^5 Da (P1) and 9.8×10^3 Da (P2)	Glucose and D-galactose with minor amounts of rhamnose and galacturonic acid	Antioxidant activity	Inturri et al. (2017)
Weissella spp.	*W. confusa* OF126	Ogi	3.00 g/L	1.1×10^6 Da	Glucose units	Antioxidant activity	Adesulu-Dahunsi et al. (2018)
	W. cibaria SJ14	Sichuan paocai	331.47 mg/L	EPS 1–7.12×10^4 Da	Mannose, glucose, galactose, arabinose, xylose, and rhamnose	Antioxidant activity	Zhu et al. (2018)
				EPS 3–3.01×10^4 Da	Galactose, mannose, glucose, and arabinose	Antioxidant activity	Zhu et al. (2018)
	W. confusa VP30	Young children's feces	59.99 ± 0.91 g/L	3.8×10^6 Da	Glucose units, glycosidic bonds	Food industry	Jin et al. (2019)
Leuconostoc spp.	*L. citreum* L3C1E7	Pico cheese	520 mg/mL	588.9×10^6 Da	Glucose units	Food industry	Domingos-Lopes, Lamosa, Stanton, Ross, and Silva (2018)
	L. mesenteroides	Chinese Sauerkraut	71.23 ± 2.25 g/L	8.79×10^7 Da	Glucopyranose	Food industry	Du et al. (2017)
Bacillus spp.	*B. sonorensis* MJM60135	Ganjang (fermented soy sauce)	8.4 ± 0.8 g/L	2.8×10^5 Da (P1) and 9.8×10^3 Da (P2)	Mannose and glucose	Food industry	Palaniyandi, Damodharan, Suh, and Yang (2018)
	B. tequilensis FR9	Native chicken intestine	6.83 g/L	2.03×10^6 Da	Glucose, galactose, mannose, arabinose, and xylose	Food industry	Rani et al. (2017)

α-1,3-linked glucose residues, for example, dextrans, produced by *Leuconostoc mesenteroides* subsp. *mesenteroides* and *Leuconostoc mesenteroides* subsp. *dextranicum* and mutans produced by *Streptococcus mutans* and *Streptococcus sobrinus*; and (2) fructans composed of β-2,6-linked fructose molecules, such as levan produced by *Streptococcus salivarius* (Cerning, 1990).

The chemical composition of HePS shows great variability in structure and consists of multiple repeats of oligosaccharides comprising 3–7 sugar residues (Laws et al., 2001). HePS are polymerized contain varying combinations with ratios of D-glucose, D-galactose, and L-rhamnose (De Vuyst & Degeest, 1999). HePS-producing strains of *Streptococcus thermophilus*, *Lactococcus lactis*, *Lactobacillus delbrueckii*, and *Lactobacillus helveticus*, among others have been identified (Evivie et al., 2017).

(Badel et al., 2011) reported that the HoPS secreted by probiotic strains are polymeric form of one type of monosaccharide unit, either glucose-based polymers (glucans) or fructose-based polymers (fructans). They exhibit molecular weights ranging from 4×10^4 to 2×10^7 Da. Within HoPS, the glucose or fructose monomers are bound by glycosidic bonds, such as a 1–6, 1–3, and 2–6 linkages. Küçükaşik et al. (2011) found *Bacillus* producing a high-molecular-weight acidic HePS containing galactose, mannose, rhamnose, and glucuronic acid. Capek et al. (2011) determined the presence of HoPS, glucan produced by *Leuconostoc garium* which contain 95% of α-(1→6) glucopyranose linkage carrying low content branches of α-(1→2), α-(1→3), and α-(1→4) linkages.

HePS and HoPS are demonstrate great variability in their structure which is made up of multiple repeats of oligosaccharides. These oligosaccharide precursors are containing varying combinations and ratios of D-glucose, L-rhamnose, D-galactose and occasionally include: D-mannose; amino-sugars. Despite their heterogenic composition, they exhibit smaller variability in size, ranging from 1×10^4 to 6×10^6 Da (Badel et al., 2011; Li et al., 2014).

16 Biosynthesis and genetics of EPS

Increasing significance of EPSs in industrial and medical biotechnology calls for the elucidation of the interrelations between metabolic pathways and biosynthesis mechanism in order to control and hence improve microbial productivity. The biosynthesis pathways of microbial EPS offer a good opportunity to produce tailor-made biogenic polymers with specific functions. Therefore, keen interest has been dedicated to understand bacterial EPS's biosynthesis mechanism and pathways and to enhance productivity in recent years (Ates, 2015).

At present, four general mechanisms are known for the production of these carbohydrate polymers in probiotic bacteria and presented in Fig. 19.2: (1) Wzx/Wzy-dependent pathway; (2) ABC transporter-dependent pathway; (3) synthase-dependent pathway; and (4) extracellular synthesis by use of a single sucrase protein. In one bacterial species, two or more pathways can coexist for the production of different macromolecules (Schmid et al., 2015). The majority of cell-surface polysaccharides are produced via the Wzx/Wzy-dependent assembly pathway; these glycans include EPSs, spore coats,

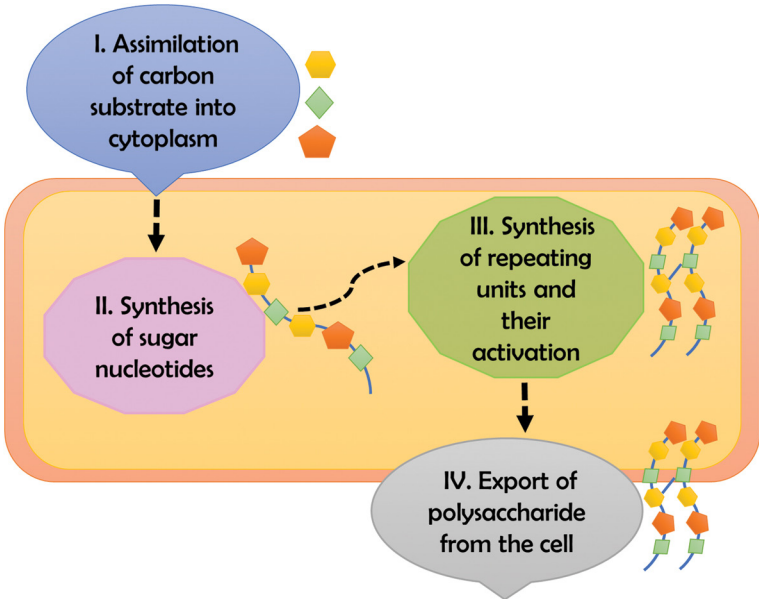


FIGURE 19.2 Four main steps involved in EPS biosynthesis.

and capsules in diverse bacteria. The key components of this assembly pathway are the integral inner membrane Wzx flippase, Wzy polymerase, and Wzz chain-length regulator proteins, which until recently have resisted detailed structural and functional characterization (Islam & Lam, 2014).

EPS operons encompass several genes with specific functions including both regulatory factors and enzymes involved in EPS biosynthesis, polymerization, and secretion, together with glycosyltransferases, which are essential for assembly of the typical EPS-repeating unit. The genetic locus that encodes enzymes required for EPS production in LAB was first identified in *S. thermophilus* and *L. lactis* (van Kranenburg et al., 1997). The metabolic products of these additional biosynthetic gene clusters were unknown, but bioinformatics-based predictions suggested that several are likely to encode products with novel structures. Moreover, the overproduction of EPSs from food grade bacteria is required to meet the market demand as effective thickeners and food ingredients. Hence, we need to understand the physiology and genetic organization of EPS-producing bacteria to overproduce the EPS by genetic modifications.

17 EPS production

Successful EPS production with higher amount is determined by the use of a suitable culture medium and cultural conditions for ex situ production. This idea not only allows high-production yields but also avoid interference of medium components during detection and quantification (Leroy & De Vuyst, 2016). EPS production is generally based on high carbon and low nitrogen substrate ratio because LAB and other probiotic bacteria are relatively fastidious microorganisms. Therefore, complex, nutrient-rich cultural media, which include yeast extract and peptones as nitrogen sources like de Man, Rogosa, Sharpe (MRS) medium (modified form) or tryptone-proteose peptone-yeast extract media, are used to grow EPS-producing probiotic strain (Guo et al., 2013; Prasanna et al., 2014).

Complex media composition increases the production of EPS, which indicates the necessity of rich nutrients like peptone, yeast extract, and beef extract for the biomass production. These media can be optimized for higher production by analyzing effects of various components on EPS yields, including the addition of several carbon and nitrogen sources and mineral salts as well (Polak-Bereck et al., 2013). The presence of levan, noncharged extracellular polysaccharide in *B. subtilis*, was identified when grown in batch cultures in sucrose rich growth medium (Dogsa et al., 2013). However, no unique and particular carbon source is available for optimal EPS yield because it is relatively a strain-dependent property that highly depends on variations in enzyme activities and carbohydrate transport systems (Leroy & De Vuyst, 2016). *Lactobacilli* spp. strains isolated from different intestinal segments of indigenous poultry noted to exhibit stronger mucoid phenotype on MRS agar supplemented with 4% (w/v) sucrose (Trabelsi et al., 2015).

On the other hand, the major issue in utilization of EPSs in commercial products is the low level of production by the majority of probiotic strains (Badel et al., 2011). EPS production of *Bacillus circulans* at all stages of culture growth and reached maximum of 0.065 mg/mL by 96 h (Vidhyalakshmi et al., 2016). To overcome this, adjustment of other process parameters such as temperature, agitation rate, and pH during fermentation must be considered for better EPS yield (Yilmaz et al., 2015). This kind of parameter adjustments not only affects bacterial growth but can also increase EPS yield like *Lactobacillus rhamnosus* in nutrient-optimized MRS broth (Polak-Berecka et al., 2014).

18 Physicochemical properties of EPS

The properties of EPS depend on their primary structure, sugar composition, and molecular mass (Trabelsi et al., 2015). Crude polysaccharides from *L. helveticus* was extracted using ion-exchange chromatography to obtain purified fractions of EPS-1, EPS-2, and EPS-3, which showed molecular weight of about 2×10^5 Da, which was composed of glucose, galactose, and mannose with a molar ratio of 1.00:1.43:9.34, 1.33:2.75:1.00, and 1.17:1.00:2.96, respectively. (Li et al., 2014). This finding also confirmed the presence of flexible chains, formed as disordered random coils in water. The extended polymer chains (fibrous morphology) and circular side chains were also clearly observed by them and concluded that the extracted EPS had branched structure.

EPS produced by *B. licheniformis* was amorphous in nature with 0.397 crystallinity index (CI_{xrd}) and identified as a levan (Singh et al., 2011). Low-intensity peak was observed, Nuclear Magnetic Resonance (NMR) spectroscopy showed the presence of α-(1→3) linkage, and single peak in the anomeric proton signal (at δ5.020 ppm) indicated the presence of α-(1→6)-linked glucan (Saravanan & Shetty, 2016).

Pyrolysis temperature and combustion of EPS was determined under atmospheric pressure to determine thermal characterization that has a significant commercial value in industrial application (Venkatesh et al., 2016). The degradation temperatures of EPS from *L. plantarum*, Lactobacillus *kefiranofaciens* locust gum and xanthan were 278.53°C–299.62°C and 278.46°C–282.65°C, respectively (Wang et al., 2010; Ahmed et al., 2013). Moreover, the degradation temperature (T_d) of

EPS extracted from *L. plantarum* was 287.7°C, and thermogravimetric mass loss spectrum for the strain EPS was between 200°C and 300°C (Wang et al., 2015).

19 Biological properties of bacterial EPS

Various biological properties of probiotic EPS have previously been studied and described in the literature because of its unique and diverse structures. Some of the biological activities and their potential beneficial benefits are discussed in this section.

20 Immunostimulatory activity

Research benefits of probiotic EPS on modulation of immune system have received the most attention among many other benefits linked with consumption of probiotics. Many evidences are available to suggest that immunomodulatory activity of a probiotic EPS is associated with its physicochemical properties. EPSs of *L. delbrueckii* exhibited antiinflammatory potential by downregulating expression of enterotoxic *Escherichia coli*-induced proinflammatory cytokines (IL-6, IL-8, and MCP-1) in porcine intestinal epithelial cells (Wachi et al., 2014). In another study, a systemic administration of *L. rhamnosus*, EPS was performed and reported a significant decrease in serum concentration of type II collagen-specific IgG antibodies in a murine model of mild rheumatoid arthritis (Nowak et al., 2012).

Through many evidences, probiotic EPSs are able to interact with host immune cells and thereby possess some positive immunomodulatory abilities. However, the molecular mechanisms responsible for these effects and their participation in immune homeostasis are yet to be studied. It has been suggested that probiotic EPS could exert their immunomodulatory capability through "mannose receptor" (Chabot et al., 2001). But the possibility of this action is still questionable because of various structural heterogeneity exhibited by probiotic EPS. Nonetheless, extremely multifunctional toll-like receptor family, which are able to identify various range of probiotic EPS may serve as the most promising vision (Patten & Collett, 2013).

21 Antioxidant property

Oxidative stress represents an imbalance between reactive oxygen species (ROS) production and ability of biological system to detoxify the reactive intermediates or to repair the caused damage. ROS, superoxide free radicals, and oxygen-derived hydroxyl are more reactive molecules and responsible for many diseases like ageing, atherosclerosis, cancer, inflammation, lung injury, Parkinson's disease, and Alzheimer's disease (Zhang et al., 2013; Zhou et al., 2016). Synthetic antioxidants were reported to be effective to slow down oxidation process, but their toxicity and safety were of great concern. Nowadays, natural antioxidants are receiving much attention from researchers and public because of their role in maintenance of human health: prevention and treatment of diseases. Hence, many researchers have suggested that polysaccharides as promising antioxidants for development of effective and nontoxic medicines (Pan & Mei, 2010; Kanmani et al., 2011).

Chiang and Pan (2012) investigated antioxidant activity of two EPS isolated from *Lactobacillus paracasei*. Both EPS showed antioxidant activity with both exhibiting comparable levels of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging to that of vitamin C used as positive control. In addition, both EPS exhibited ferrous iron chelation and moderate lipid peroxidation when compared to vitamin C and Ethylenediaminetetraacetic acid (EDTA) controls. Similarly, EPS isolated from *L. rhamnosus* (Polak-Berecka et al., 2013), *L. plantarum* (Li et al., 2013; Zhang et al., 2013), and *L. helveticus* (Li et al., 2014) have exhibited moderate free radical scavenging and metal ion chelating activities. EPS extracted from *B. circulans* exhibited dose-dependent DPPH scavenging activity showing 97% antioxidant activity at 1000 µg/mL (Vidhyalakshmi et al., 2016).

22 Anticancer effects

Due to vast effects of chemotherapy treatments for cancer, antitumor effects of naturally produced alternatives like probiotic EPS are receiving increased attention in research. In a recent study, anticancer effect has been explored in EPS of four *Lactobacillus* sp. isolated from healthy infant feces and yoghurt (Tukenmez et al., 2019). They studied relationship between the capability of *Lactobacillus* EPS to induce apoptosis and their monosaccharide composition, molecular weight, and linkage type. *Lactobacillus* EPS were able to inhibit the proliferation in a time-dependent manner and induced apoptosis via increasing the expression of Bax, Caspase 3 and 9 while decreasing Bcl-2 and survivin. In the same study, they reported that

the EPS of *L. delbrueckii* ssp. *bulgaricus* suggested that the ability of EPS to induce apoptosis might be due to its mannose and glucose composition (Tukenmez et al., 2019).

Wang et al. (2019) isolated a new *Bifidobacterium* strain, named *Bifidobacterium breve*, from infant feces. They found that *B. breve* harbors a new EPS-encoding cluster with 14 predicted genes, which could be divided into three groups according to biosynthesis pathway hypothesis. Furthermore, they reported its anticancer activity against head and neck squamous cell carcinoma line by regulating cell cycle arrest and cell apoptosis promotion (Wang et al., 2019).

23 Cholesterol-lowering activity

Pharmacological agents that effectively lower cholesterol levels are available for the treatment of hypercholesterolemia; however, they are very expensive and are known to exhibit a wide range of side effects. Several researches have indicated that the consumption of certain cultured dairy products reduce serum cholesterol (Gilliland et al 1985; Nakajima et al., 1992). Hence, interest in the use of probiotics for lowering blood cholesterol levels has been increasing in therapeutic practice. Probiotics producing EPS have been suggested to lower cholesterol levels by binding the cholesterol to bacterial cells through EPS. The cholesterol removal mechanism by binding or adhering to the probiotic cells, especially to the EPS and surrounding the probiotic cells as a capsule, has potential significance in control of serum cholesterol concentration in humans (Tok & Aslim, 2010).

In a study, Nakajima et al. (1992) reported the cholesterol-lowering activity of milk fermented with an EPS-producing lactic acid bacterium. This investigation suggested EPS has a potential to interfere with absorption of cholesterol, or of bile acids, from intestines by binding and removing them from body. EPS produced by *L. kefiranofaciens* could reduce serum cholesterol levels as well as suppress the increase in blood pressure (Maeda et al., 2004). Hence, the probiotic strain that has high EPS production capacity could be used to manufacture a fermented dairy product which could enhance the anticholesterolemic activity.

24 Conclusion

Overall, the studies on production of bacteriocins and EPSs from probiotic strains may lead to the production of highly selective probioactives. However, it is still necessary to explore the interaction behavior of these probioactives with human GIT in order to understand their actions and nature of resulting metabolites excreted by them. Based on the antimicrobial scenario of probiotic bacteriocin, it is possible that these bacteriocins might have multiple activities, namely, anticancer properties like nisin. Therefore, probioactives like bacteriocins necessarily require an intensified and well-designed quality-focused research to bring out the novel functional attributes and develop them as promising clinical therapeutic agents for human and animal use. The novel functionalities of probiotic EPSs in several fields like food, medical, pharmaceutical products, and cosmetics made their applicability to several other fields become practically limitless. Therefore, rapid expansion in the search of novel EPS-producing probiotic strain and optimization of fermentation conditions would lead to vital success with the extensive scientific outputs.

References

Abriouel, H., Franz, C. M., Ben Omar, N., & Galvez, A. (2011). Diversity and applications of *Bacillus* bacteriocins. *FEMS Microbiology Reviews*, *35*(1), 201–232. doi: 10.1111/j.1574-6976.2010.00244.x.

Adesulu-Dahunsi, A. T., Sanni, A. I., Jeyaram, K., Ojediran, J. O., Ogunsakin, A. O., & Banwo, K. (2018). Extracellular polysaccharide from *Weissella confusa* OF126: Production, optimization, and characterization. *International Journal of Biological Macromolecules*, *111*, 514–525. doi: 10.1016/j.ijbiomac.2018.01.060.

Ahmed, Z., Wang, Y., Anjum, N., Ahmad, H., Ahmad, A., & Raza, M. (2013). Characterization of new exopolysaccharides produced by coculturing of *L. kefiranofaciens* with yoghurt strains. *International Journal of Biological Macromolecules*, *59*(Suppl. C), 377–383. doi: 10.1016/j.ijbiomac.2013.04.075.

Argyri, A. A., Zoumpopoulou, G., Karatzas, K. A., Tsakalidou, E., Nychas, G. J., Panagou, E. Z., et al. (2013). Selection of potential probiotic lactic acid bacteria from fermented olives by in vitro tests. *Food Microbiology*, *33*(2), 282–291. doi: 10.1016/j.fm.2012.10.005.

Ates, O. (2015). Systems biology of microbial exopolysaccharides production. *Frontiers in Bioengineering and Biotechnology*, *3*, 200. doi: 10.3389/fbioe.2015.00200.

Babasaki, K., Takao, T., Shimonishi, Y., & Kurahashi, K. (1985). Subtilosin A, a new antibiotic peptide produced by *Bacillus subtilis* 168: Isolation, structural analysis, and biogenesis. *Journal of Biochemistry*, *98*(3), 585–603. doi: 10.1093/oxfordjournals.jbchem.a135315.

Badel, S., Bernardi, T., & Michaud, P. (2011). New perspectives for *Lactobacilli* exopolysaccharides. *Biotechnology Advances*, *29*(1), 54–66. doi: 10.1016/j.biotechadv.2010.08.011.

Baindara, P., Korpole, S., & Grover, V. (2018). Bacteriocins: Perspective for the development of novel anticancer drugs. *Applied Microbiology and Biotechnology*, *102*(24), 10393–10408. doi: 10.1007/s00253-018-9420-8.

Balzaretti, S., Taverniti, V., Guglielmetti, S., Fiore, W., Minuzzo, M., Ngo, H. N., et al. (2017). A novel rhamnose-rich hetero-exopolysaccharide isolated from *Lactobacillus paracasei* DG activates THP-1 human monocytic cells. *Applied and Environmental Microbiology*, *83*(3), e02702–e02716. doi: 10.1128/aem.02702-16.

Beric, T., Stankovic, S., Draganic, V., Kojic, M., Lozo, J., & Fira, D. (2014). Novel antilisterial bacteriocin licheniocin 50.2 from *Bacillus licheniformis* VPS50.2 isolated from soil sample. *Journal of Applied Microbiology*, *116*(3), 502–510. doi: 10.1111/jam.12393.

Birri, D. J., Brede, D. A., Forberg, T., Holo, H., & Nes, I. F. (2010). Molecular and genetic characterization of a novel bacteriocin locus in *Enterococcus avium* isolates from infants. *Applied and Environmental Microbiology*, *76*(2), 483–492. doi: 10.1128/AEM.01597-09.

Cani, P. D. (2018). Human gut microbiome: Hopes, threats and promises. *Gut*, *67*(9), 1716–1725. doi: 10.1136/gutjnl-2018-316723.

Capek, P., Hlavoňová, E., Matulová, M., Mislovicová, D., Růžička, J., Koutný, M., et al. (2011). Isolation and characterization of an extracellular glucan produced by *Leuconostoc garlicum* PR. *Carbohydrate Polymers*, *83*(1), 88–93. doi: 10.1016/j.carbpol.2010.07.024.

Carr, F. J., Chill, D., & Maida, N. (2002). The lactic acid bacteria: A literature survey. *Critical Reviews in Microbiology*, *28*(4), 281–370. doi: 10.1080/1040-840291046759.

Cerning, J. (1990). Exocellular polysaccharides produced by lactic acid bacteria. *FEMS Microbiology Reviews*, *7*(1–2), 113–130. doi: 10.1111/j.1574-6968.1990.tb04883.x.

Chabot, S., Yu, H. -L., Léséleuc, L. D., Cloutier, D., Calsteren, M. -R. V., Lessard, M., et al. (2001). Exopolysaccharides from *Lactobacillus rhamnosus* RW-9595M stimulate TNF. *Lait*, *81*(6), 683–697.

Champagne, C. P., Gomes da Cruz, A., & Daga, M. (2018). Strategies to improve the functionality of probiotics in supplements and foods. *Current Opinion in Food Science*, *22*, 160–166. doi: 10.1016/j.cofs.2018.04.008.

Cherif, A., Rezgui, W., Raddadi, N., Daffonchio, D., & Boudabous, A. (2008). Characterization and partial purification of entomocin 110, a newly identified bacteriocin from *Bacillus thuringiensis* subsp. *Entomocidus* HD110. *Microbiology Research*, *163*(6), 684–692. doi: 10.1016/j.micres.2006.10.005.

Chiang, S. S., & Pan, T. M. (2012). Beneficial effects of L*actobacillus paracasei* subsp. *paracasei* NTU 101 and its fermented products. *Applied Microbiology and Biotechnology*, *93*(3), 903–916. doi: 10.1007/s00253-011-3753-x.

Chong, E. S. L. (2014). A potential role of probiotics in colorectal cancer prevention: Review of possible mechanisms of action. *World Journal of Microbiology and Biotechnology*, *30*(2), 351–374. doi: 10.1007/s11274-013-1499-6.

Chopra, L., Singh, G., Choudhary, V., & Sahoo, D. K. (2014). Sonorensin: An antimicrobial peptide, belonging to the heterocycloanthracin subfamily of bacteriocins, from a new marine isolate, *Bacillus sonorensis* MT93. *Applied and Environmental Microbiology*, *80*(10), 2981–2990. doi: 10.1128/AEM.04259-13.

Collado, M. C., Meriluoto, J., & Salminen, S. (2008). Adhesion and aggregation properties of probiotic and pathogen strains. *European Food Research and Technology*, *226*(5), 1065–1073. doi: 10.1007/s00217-007-0632-x.

Conlon, M. A., & Bird, A. R. (2014). The impact of diet and lifestyle on gut microbiota and human health. *Nutrients*, *7*(1), 17–44. doi: 10.3390/nu7010017.

Corzo, G., & Gilliland, S. E. (1999). Bile salt hydrolase activity of three strains of *Lactobacillus* acidophilus. *Journal of Dairy Science*, *82*(3), 472–480. doi: 10.3168/jds.S0022-0302(99)75256-2.

Cotter, P. D., Ross, R. P., & Hill, C. (2013). Bacteriocins—A viable alternative to antibiotics? *Nature Reviews Microbiology*, *11*(2), 95–105. doi: 10.1038/nrmicro2937.

Das, D., & Goyal, A. (2014). Characterization of a noncytotoxic bacteriocin from probiotic *Lactobacillus plantarum* DM5 with potential as a food preservative. *Food & Function*, *5*(10), 2453–2462. doi: 10.1039/c4fo00481g.

De Vrese, M., & Schrezenmeir, J. (2008). Probiotics, prebiotics, and synbiotics. *Advances in Biochemical Engineering/Biotechnology*, *111*, 1–66. doi: 10.1007/10_2008_097.

De Vuyst, L., & Degeest, B. (1999). Heteropolysaccharides from lactic acid bacteria. *FEMS Microbiology Reviews*, *23*(2), 153–177. doi: 10.1111/j.1574-6976.1999.tb00395.x.

De Vuyst, L., & Vandamme, E. J. (1994). Lactic acid bacteria and bacteriocins: Their practical importance. In L. De Vuyst, & E. J. Vandamme (Eds.), *Bacteriocins of lactic acid bacteria: Microbiology, genetics and applications* (pp. 1–11). Boston, MA: Springer US.

Deepak, V., Ramachandran, S., Balahmar, R. M., Pandian, S. R., Sivasubramaniam, S. D., Nellaiah, H., et al. (2016). In vitro evaluation of anticancer properties of exopolysaccharides from *Lactobacillus acidophilus* in colon cancer cell lines. *In Vitro Cellular & Developmental Biology – Animal*, *52*(2), 163–173. doi: 10.1007/s11626-015-9970-3.

Dertli, E., Mayer, M. J., & Narbad, A. (2015). Impact of the exopolysaccharide layer on biofilms, adhesion and resistance to stress in *Lactobacillus johnsonii* FI9785. *BMC Microbiology*, *15*(1), 8. doi: 10.1186/s12866-015-0347-2.

Di, W., Zhang, L., Wang, S., Yi, H., Han, X., Fan, R., et al. (2017). Physicochemical characterization and antitumour activity of exopolysaccharides produced by *Lactobacillus casei* SB27 from yak milk. *Carbohydrate Polymers*, *171*, 307–315. doi: 10.1016/j.carbpol.2017.03.018.

Dobson, A., Cotter, P. D., Ross, R. P., & Hill, C. (2012). Bacteriocin production: A probiotic trait? *Applied and Environmental Microbiology*, *78*(1), 1–6. doi: 10.1128/AEM.05576-11.

Dogsa, I., Brloznik, M., Stopar, D., & Mandic-Mulec, I. (2013). Exopolymer diversity and the role of levan in *Bacillus subtilis* biofilms. *PLoS One*, *8*(4), e62044. doi: 10.1371/journal.pone.0062044.

Domingos-Lopes, M. F. P., Lamosa, P., Stanton, C., Ross, R. P., & Silva, C. C. G. (2018). Isolation and characterization of an exopolysaccharide-producing *Leuconostoc citreum* strain from artisanal cheese. *Letters in Applied Microbiology*, *67*(6), 570–578. doi: 10.1111/lam.13073.

Du, R., Xing, H., Yang, Y., Jiang, H., Zhou, Z., & Han, Y. (2017). Optimization, purification and structural characterization of a dextran produced by *L. mesenteroides* isolated from Chinese sauerkraut. *Carbohydrate Polymers*, *174*, 409–416. doi: 10.1016/j.carbpol.2017.06.084.

Egan, K., Field, D., Rea, M. C., Ross, R. P., Hill, C., & Cotter, P. D. (2016). Bacteriocins: Novel solutions to age old spore-related problems? *Frontiers in Microbiology*, *7*, 461. doi: 10.3389/fmicb.2016.00461.

Evivie, S. E., Huo, G. -C., Igene, J. O., & Bian, X. (2017). Some current applications, limitations and future perspectives of lactic acid bacteria as probiotics. *Food & Nutrition Research*, *61*(1), 1318034. doi: 10.1080/16546628.2017.1318034.

Farnworth, E. R., & Champagne, C. (2010). Chapter 1 – Production of probiotic cultures and their incorporation into foods. In R. R. Watson, & V. R. Preedy (Eds.), *Bioactive foods in promoting health* (pp. 3–17). Boston, MA: Academic Press.

Fluhe, L., Knappe, T. A., Gattner, M. J., Schafer, A., Burghaus, O., Linne, U., et al. (2012). The radical SAM enzyme AlbA catalyzes thioether bond formation in subtilosin A. *Nature Chemical Biology*, *8*(4), 350–357. doi: 10.1038/nchembio.798.

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., et al. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology*, *14*(8), 491–502. doi: 10.1038/nrgastro.2017.75.

Gilliland, S. E., Nelson, C. R., & Maxwell, C. (1985). Assimilation of cholesterol by *Lactobacillus acidophilus*. *Applied and Environmental Microbiology*, *49*(2), 377–381.

Górska, A., Przystupski, D., Niemczura, M. J., & Kulbacka, J. (2019). Probiotic bacteria: A promising tool in cancer prevention and therapy. *Current Microbiology*, *76*(8), 939–949. doi: 10.1007/s00284-019-01679-8.

Guo, Y., Pan, D., Sun, Y., Xin, L., Li, H., & Zeng, X. (2013). Antioxidant activity of phosphorylated exopolysaccharide produced by *Lactococcus lactis* subsp. lactis. *Carbohydrate Polymers*, *97*(2), 849–854. doi: 10.1016/j.carbpol.2013.06.024.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., et al. (2014). Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*, *11*(8), 506–514. doi: 10.1038/nrgastro.2014.66.

Hu, M., Zhao, H., Zhang, C., Yu, J., & Lu, Z. (2013). Purification and characterization of plantaricin 163, a novel bacteriocin produced by *Lactobacillus plantarum* 163 isolated from traditional Chinese fermented vegetables. *Journal of Agricultural and Food Chemistry*, *61*(47), 11676–11682. doi: 10.1021/jf403370y.

Ibarburu, I., Puertas, A. I., Berregi, I., Rodriguez-Carvajal, M. A., Prieto, A., & Duenas, M. T. (2015). Production and partial characterization of exopolysaccharides produced by two *Lactobacillus suebicus* strains isolated from cider. *International Journal of Food Microbiology*, *214*, 54–62. doi: 10.1016/j.ijfoodmicro.2015.07.012.

Indira, M., Venkateswarulu, T. C., Abraham Peele, K., Nazneen Bobby, M., & Krupanidhi, S. (2019). Bioactive molecules of probiotic bacteria and their mechanism of action: A review. *3 Biotech*, *9*(8), 306. doi: 10.1007/s13205-019-1841-2.

Inturri, R., Molinaro, A., Di Lorenzo, F., Blandino, G., Tomasello, B., Hidalgo-Cantabrana, C., et al. (2017). Chemical and biological properties of the novel exopolysaccharide produced by a probiotic strain of *Bifidobacterium longum*. *Carbohydrate Polymers*, *174*, 1172–1180. doi: 10.1016/j.carbpol.2017.07.039.

Islam, S. T., & Lam, J. S. (2014). Synthesis of bacterial polysaccharides via the Wzx/Wzy-dependent pathway. *Canadian Journal of Microbiology*, *60*(11), 697–716. doi: 10.1139/cjm-2014-0595.

Ismail, B., & Nampoothiri, K. M. (2010). Production, purification and structural characterization of an exopolysaccharide produced by a probiotic *Lactobacillus plantarum* MTCC 9510. *Archives of Microbiology*, *192*(12), 1049–1057. doi: 10.1007/s00203-010-0636-y.

Jackson, S. A., Schoeni, J. L., Vegge, C., Pane, M., Stahl, B., Bradley, M., et al. (2019). Improving end-user trust in the quality of commercial probiotic products. *Frontiers in Microbiology*, *10*, 739. doi: 10.3389/fmicb.2019.00739.

Jeong, S. Y., Park, S. Y., Kim, Y. H., Kim, M., & Lee, S. J. (2008). Cytotoxicity and apoptosis induction of *Bacillus vallismortis* BIT-33 metabolites on colon cancer carcinoma cells. *Journal of Applied Microbiology*, *104*(3), 796–807. doi: 10.1111/j.1365-2672.2007.03615.x.

Jimenez, J. J., Diep, D. B., Borrero, J., Gutiez, L., Arbulu, S., Nes, I. F., et al. (2015). Cloning strategies for heterologous expression of the bacteriocin enterocin A by *Lactobacillus sakei* Lb790, *Lb. plantarum* NC8 and *Lb. casei* CECT475. *Microbial Cell Factories*, *14*, 166. doi: 10.1186/s12934-015-0346-x.

Jin, H., Jeong, Y., Yoo, S. -H., Johnston, T. V., Ku, S., & Ji, G. E. (2019). Isolation and characterization of high exopolysaccharide-producing *Weissella confusa* VP30 from young children's feces. *Microbial Cell Factories*, *18*(1), 110. doi: 10.1186/s12934-019-1158-1.

Kanmani, P., Satish Kumar, R., Yuvaraj, N., Paari, K. A., Pattukumar, V., & Arul, V. (2011). Production and purification of a novel exopolysaccharide from lactic acid bacterium *Streptococcus phocae* PI80 and its functional characteristics activity in vitro. *Bioresource Technology*, *102*(7), 4827–4833. doi: 10.1016/j.biortech.2010.12.118.

Kareb, O., & Aider, M. (2020). Quorum sensing circuits in the communicating mechanisms of bacteria and its implication in the biosynthesis of bacteriocins by lactic acid bacteria: A review. *Probiotics and Antimicrobial Proteins*, *12*(1), 5–17. doi: 10.1007/s12602-019-09555-4.

Kasuga, G., Tanaka, M., Harada, Y., Nagashima, H., Yamato, T., Wakimoto, A., et al. (2019). Homologous expression and characterization of gassericin T and gassericin S, a novel class iib bacteriocin produced by *Lactobacillus gasseri* LA327. *Applied and Environmental Microbiology*, *85*(6), e02815–18. doi: 10.1128/AEM.02815-18.

Kawai, Y., Ishii, Y., Uemura, K., Kitazawa, H., Saito, T., & Itoh, T. (2001). *Lactobacillus reuteri* LA6 and *Lactobacillus gasseri* LA39 isolated from faeces of the same human infant produce identical cyclic bacteriocin. *Food Microbiology*, *18*(4), 407–415. doi: 10.1006/fmic.2001.0412.

Kawai, Y., Saito, T., Kitazawa, H., & Itoh, T. (1998). Gassericin A; an uncommon cyclic bacteriocin produced by *Lactobacillus gasseri* LA39 linked at N- and C-terminal ends. *Bioscience, Biotechnology, and Biochemistry*, *62*(12), 2438–2440. doi: 10.1271/bbb.62.2438.

Kawai, Y., Saito, T., Toba, T., Samant, S. K., & Itoh, T. (1994). Isolation and characterization of a highly hydrophobic new bacteriocin (gassericin A) from *Lactobacillus gasseri* LA39. *Bioscience, Biotechnology, and Biochemistry*, *58*(7), 1218–1221. doi: 10.1271/bbb.58.1218.

Kawai, Y., Saitoh, B., Takahashi, O., Kitazawa, H., Saito, T., Nakajima, H., et al. (2000). Primary amino acid and DNA sequences of gassericin T, a lactacin F-family bacteriocin produced by *Lactobacillus gasseri* SBT2055. *Bioscience, Biotechnology, and Biochemistry*, *64*(10), 2201–2208. doi: 10.1271/bbb.64.2201.

Kawulka, K. E., Sprules, T., Diaper, C. M., Whittal, R. M., McKay, R. T., Mercier, P., et al. (2004). Structure of subtilosin A, a cyclic antimicrobial peptide from *Bacillus subtilis* with unusual sulfur to alpha-carbon cross-links: Formation and reduction of alpha-thio-alpha-amino acid derivatives. *Biochemistry*, *43*(12), 3385–3395. doi: 10.1021/bi0359527.

Khochamit, N., Siripornadulsil, S., Sukon, P., & Siripornadulsil, W. (2015). Antibacterial activity and genotypic-phenotypic characteristics of bacteriocin-producing *Bacillus subtilis* KKU213: Potential as a probiotic strain. *Microbiological Research*, *170*, 36–50. doi: 10.1016/j.micres.2014.09.004.

Küçükaşik, F., Kazak, H., Güney, D., Finore, I., Poli, A., Yenigün, O., et al. (2011). Molasses as fermentation substrate for levan production by *Halomonas* sp.. *Applied Microbiology and Biotechnology*, *89*(6), 1729–1740. doi: 10.1007/s00253-010-3055-8.

Lavilla-Lerma, L., Perez-Pulido, R., Martinez-Bueno, M., Maqueda, M., & Valdivia, E. (2013). Characterization of functional, safety, and gut survival related characteristics of *Lactobacillus* strains isolated from farmhouse goat's milk cheeses. *International Journal of Food Microbiology*, *163*(2–3), 136–145. doi: 10.1016/j.ijfoodmicro.2013.02.015.

Laws, A., Gu, Y., & Marshall, V. (2001). Biosynthesis, characterisation, and design of bacterial exopolysaccharides from lactic acid bacteria. *Biotechnology Advances*, *19*(8), 597–625. doi: 10.1016/s0734-9750(01)00084-2.

Lebeer, S., Vanderleyden, J., & De Keersmaecker, S. C. J. (2010). Host interactions of probiotic bacterial surface molecules: Comparison with commensals and pathogens. *Nature Reviews Microbiology*, *8*(3), 171–184. doi: 10.1038/nrmicro2297.

Leclerc, P. L., Gauthier, S. F., Bachelard, H., Santure, M., & Roy, D. (2002). Antihypertensive activity of casein-enriched milk fermented by *Lactobacillus* helveticus. *International Dairy Journal*, *12*(12), 995–1004. doi: 10.1016/s0958-6946(02)00125-5.

Leroy, F., & De Vuyst, L. (2016). Advances in production and simplified methods for recovery and quantification of exopolysaccharides for applications in food and health. *Journal of Dairy Science*, *99*(4), 3229–3238. doi: 10.3168/jds.2015-9936.

Li, J. -Y., Jin, M. -M., Meng, J., Gao, S. -M., & Lu, R. -R. (2013). Exopolysaccharide from *Lactobacillus plantarum* LP6: Antioxidation and the effect on oxidative stress. *Carbohydrate Polymers*, *98*(1), 1147–1152. doi: 10.1016/j.carbpol.2013.07.027.

Li, W., Ji, J., Chen, X., Jiang, M., Rui, X., & Dong, M. (2014). Structural elucidation and antioxidant activities of exopolysaccharides from *Lactobacillus helveticus* MB2-1. *Carbohydrate Polymer*, *102*, 351–359. doi: 10.1016/j.carbpol.2013.11.053.

Liu, X., Lee, J. Y., Jeong, S. J., Cho, K. M., Kim, G. M., Shin, J. H., et al. (2015). Properties of a bacteriocin produced by *Bacillus subtilis* EMD4 Isolated from Ganjang (soy sauce). *Journal Microbiology Biotechnology*, *25*(9), 1493–1501. doi: 10.4014/jmb.1502.02037.

Liu, Z., Zhang, Z., Qiu, L., Zhang, F., Xu, X., Wei, H., et al. (2017). Characterization and bioactivities of the exopolysaccharide from a probiotic strain of *Lactobacillus plantarum* WLPL04. *Journal of Dairy Science*, *100*(9), 6895–6905. doi: 10.3168/jds.2016-11944.

Maeda, H., Zhu, X., Omura, K., Suzuki, S., & Kitamura, S. (2004). Effects of an exopolysaccharide (kefiran) on lipids, blood pressure, blood glucose, and constipation. *Biofactors*, *22*(1–4), 197–200. doi: 10.1002/biof.5520220141.

Maldonado-Barragan, A., Caballero-Guerrero, B., Martin, V., Ruiz-Barba, J. L., & Rodriguez, J. M. (2016). Purification and genetic characterization of gassericin E, a novel co-culture inducible bacteriocin from *Lactobacillus gasseri* EV1461 isolated from the vagina of a healthy woman. *BMC Microbiology*, *16*, 37. doi: 10.1186/s12866-016-0663-1.

Marx, R., Stein, T., Entian, K. D., & Glaser, S. J. (2001). Structure of the *Bacillus subtilis* peptide antibiotic subtilosin A determined by 1H-NMR and matrix assisted laser desorption/ionization time-of-flight mass spectrometry. *Journal Protein Chemistry*, *20*(6), 501–506. doi: 10.1023/a:1012562631268.

Messaoudi, S., Madi, A., Prevost, H., Feuilloley, M., Manai, M., Dousset, X., et al. (2012). In vitro evaluation of the probiotic potential of *Lactobacillus salivarius* SMXD51. *Anaerobe*, *18*(6), 584–589. doi: 10.1016/j.anaerobe.2012.10.004.

Mouloud, G., Daoud, H., Bassem, J., Laribi Atef, I., & Hani, B. (2013). New bacteriocin from *Bacillus clausii* strainGM17: Purification, characterization, and biological activity. *Applied Biochemistry and Biotechnology*, *171*(8), 2186–2200. doi: 10.1007/s12010-013-0489-3.

Mukherjee, S., & Ramesh, A. (2015). Bacteriocin-producing strains of *Lactobacillus plantarum* inhibit adhesion of *Staphylococcus aureus* to extracellular matrix: Quantitative insight and implications in antibacterial therapy. *Journal of Medical Microbiology*, *64*(12), 1514–1526. doi: 10.1099/jmm.0.000181.

Nakajima, H., Suzuki, Y., & Hirota, T. (1992). Cholesterol lowering activity of ropy fermented milk. *Journal of Food Science*, *57*(6), 1327–1329. doi: 10.1111/j.1365-2621.1992.tb06848.x.

Nowak, B., Ciszek-Lenda, M., Srottek, M., Gamian, A., Kontny, E., Gorska-Fraczek, S., et al. (2012). *Lactobacillus rhamnosus* exopolysaccharide ameliorates arthritis induced by the systemic injection of collagen and lipopolysaccharide in DBA/1 mice. *Archivum Immunologiae et Therapiae Experimentalis*, *60*(3), 211–220. doi: 10.1007/s00005-012-0170-5.

O'Connor, P. M., Kuniyoshi, T. M., Oliveira, R. P., Hill, C., Ross, R. P., & Cotter, P. D. (2020). Antimicrobials for food and feed; a bacteriocin perspective. *Current Opinion in Biotechnology*, *61*, 160–167. doi: 10.1016/j.copbio.2019.12.023.

Paiva, A. D., de Oliveira, M. D., de Paula, S. O., Baracat-Pereira, M. C., Breukink, E., & Mantovani, H. C. (2012). Toxicity of bovicin HC5 against mammalian cell lines and the role of cholesterol in bacteriocin activity. *Microbiology*, *158*(11), 2851–2858. doi: 10.1099/mic.0.062190-0.

Palaniyandi, S. A., Damodharan, K., Suh, J. -W., & Yang, S. H. (2018). Functional characterization of an exopolysaccharide produced by *Bacillus sonorensis* MJM60135 isolated from Ganjang. *Journal of Microbiology and Biotechnology*, *28*(5), 663–670. doi: 10.4014/jmb.1711.11040.

Pan, D., & Mei, X. (2010). Antioxidant activity of an exopolysaccharide purified from *Lactococcus lactis* subsp. lactis 12. *Carbohydrate Polymers*, *80*(3), 908–914. doi: 10.1016/j.carbpol.2010.01.005.

Pandey, N., Malik, R. K., Kaushik, J. K., & Singroha, G. (2013). Gassericin A: A circular bacteriocin produced by lactic acid bacteria *Lactobacillus gasseri*. *World Journal of Microbiology & Biotechnology*, *29*(11), 1977–1987. doi: 10.1007/s11274-013-1368-3.

Parveen Rani, R., Anandharaj, M., Hema, S., Deepika, R., & David Ravindran, A. (2016). Purification of antilisterial peptide (Subtilosin A) from novel *Bacillus tequilensis* FR9 and demonstrate their pathogen invasion protection ability using human carcinoma cell line. *Frontiers in Microbiology*, *7*, 1910. doi: 10.3389/fmicb.2016.01910.

Patten, D. A., & Collett, A. (2013). Exploring the immunomodulatory potential of microbial-associated molecular patterns derived from the enteric bacterial microbiota. *Microbiology*, *159*(Pt_8), 1535–1544. doi: 10.1099/mic.0.064717-0.

Perez, K. J., Viana, J. D. S., Lopes, F. C., Pereira, J. Q., Dos Santos, D. M., Oliveira, J. S., et al. (2017). *Bacillus* spp. Isolated from Puba as a source of biosurfactants and antimicrobial lipopeptides. *Frontiers in Microbiology*, *8*, 61. doi: 10.3389/fmicb.2017.00061.

Polak-Berecka, M., Wasko, A., & Kubik-Komar, A. (2014). Optimization of culture conditions for exopolysaccharide production by a probiotic strain of *Lactobacillus rhamnosus* E/N. *Polish Journal of Microbiology*, *63*(2), 253–257.

Polak-Berecka, M., Waśko, A., Szwajgier, D., & Chomaz, A. (2013). Bifidogenic and antioxidant activity of exopolysaccharides produced by *Lactobacillus rhamnosus* E/N cultivated on different carbon sources. *Polish Journal of Microbiology*, *62*(2), 181–188.

Poole, J., Day, C. J., von Itzstein, M., Paton, J. C., & Jennings, M. P. (2018). Glycointeractions in bacterial pathogenesis. *Nature Reviews Microbiology*, *16*(7), 440–452. doi: 10.1038/s41579-018-0007-2.

Prasanna, P. H. P., Grandison, A. S., & Charalampopoulos, D. (2014). Bifidobacteria in milk products: An overview of physiological and biochemical properties, exopolysaccharide production, selection criteria of milk products and health benefits. *Food Research International*, *55*, 247–262. doi: 10.1016/j.foodres.2013.11.013.

Rahbar Saadat, Y., Yari Khosroushahi, A., & Pourghassem Gargari, B. (2019). A comprehensive review of anticancer, immunomodulatory and health beneficial effects of the lactic acid bacteria exopolysaccharides. *Carbohydrate Polymers*, *217*, 79–89. doi: 10.1016/j.carbpol.2019.04.025.

Rani, R. P., Anandharaj, M., & David Ravindran, A. (2018). Characterization of a novel exopolysaccharide produced by *Lactobacillus gasseri* FR4 and demonstration of its in vitro biological properties. *International Journal of Biological Macromolecules*, *109*, 772–783. doi: 10.1016/j.ijbiomac.2017.11.062.

Rani, R. P., Anandharaj, M., Sabhapathy, P., & Ravindran, A. D. (2017). Physiochemical and biological characterization of novel exopolysaccharide produced by *Bacillus* tequilensis FR9 isolated from chicken. *International Journal of Biological Macromolecules*, *96*, 1–10. doi: 10.1016/j.ijbiomac.2016.11.122.

Reddy, G., Altaf, M., Naveena, B. J., Venkateshwar, M., & Kumar, E. V. (2008). Amylolytic bacterial lactic acid fermentation—A review. *Biotechnology Advances*, *26*(1), 22–34. doi: 10.1016/j.biotechadv.2007.07.004.

Rossi, M., Mirbagheri, S. E. Y. E. D. S., Keshavarzian, A., & Bishehsari, F. (2018). Nutraceuticals in colorectal cancer: A mechanistic approach. *European Journal of Pharmacology*, *833*, 396–402. doi: 10.1016/j.ejphar.2018.06.027.

Sabate, D. C., & Audisio, M. C. (2013). Inhibitory activity of surfactin, produced by different *Bacillus subtilis* subsp. subtilis strains, against *Listeria monocytogenes* sensitive and bacteriocin-resistant strains. *Microbiological Research*, *168*(3), 125–129. doi: 10.1016/j.micres.2012.11.004.

Sanders, M. E., Benson, A., Lebeer, S., Merenstein, D. J., & Klaenhammer, T. R. (2018). Shared mechanisms among probiotic taxa: Implications for general probiotic claims. *Current Opinion in Biotechnology*, *49*, 207–216. doi: 10.1016/j.copbio.2017.09.007.

Saravanan, C., & Shetty, P. K. (2016). Isolation and characterization of exopolysaccharide from *Leuconostoc* lactis KC117496 isolated from idli batter. *International Journal of Biological Macromolecules*, *90*, 100–106. doi: 10.1016/j.ijbiomac.2015.02.007.

Schillinger, U., & Lücke, F. K. (1989). Antibacterial activity of *Lactobacillus sake* isolated from meat. *Applied and Environmental Microbiology*, *55*(8), 1901–1906.

Schmid, J., Sieber, V., & Rehm, B. (2015). Bacterial exopolysaccharides: Biosynthesis pathways and engineering strategies. *Frontiers in Microbiology*, *6*, 496. doi: 10.3389/fmicb.2015.00496.

Shao, L., Wu, Z., Tian, F., Zhang, H., Liu, Z., Chen, W., et al. (2015). Molecular characteristics of an exopolysaccharide from *Lactobacillus rhamnosus* KF5 in solution. *International Journal of Biological Macromolecules*, *72*, 1429–1434. doi: 10.1016/j.ijbiomac.2014.10.015.

Sharma, G., Dang, S., Gupta, S., & Gabrani, R. (2018). Antibacterial activity, cytotoxicity, and the mechanism of action of bacteriocin from *Bacillus subtilis* GAS101. *Medical Principles and Practice*, *27*(2), 186–192. doi: 10.1159/000487306.

Singh, R. P., Shukla, M. K., Mishra, A., Kumari, P., Reddy, C. R. K., & Jha, B. (2011). Isolation and characterization of exopolysaccharides from seaweed associated bacteria *Bacillus licheniformis*. *Carbohydrate Polymers*, *84*(3), 1019–1026. doi: 10.1016/j.carbpol.2010.12.061.

Stein, T. (2005). *Bacillus subtilis* antibiotics: Structures, syntheses and specific functions. *Molecular Microbiology*, *56*(4), 845–857. doi: 10.1111/j.1365-2958.2005.04587.x.

Sutyak, K. E., Wirawan, R. E., Aroutcheva, A. A., & Chikindas, M. L. (2008). Isolation of the *Bacillus subtilis* antimicrobial peptide subtilosin from the dairy product-derived *Bacillus amyloliquefaciens*. *Journal of Applied Microbiology*, *104*(4), 1067–1074. doi: 10.1111/j.1365-2672.2007.03626.x.

Tahara, T., Yoshioka, S., Utsumi, R., & Kanatani, K. (1997). Isolation and partial characterization of bacteriocins produced by *Lactobacillus gasseri* JCM 2124. *FEMS Microbiology Letters*, *148*(1), 97–100. doi: 10.1111/j.1574-6968.1997.tb10273.x.

Tanaka, H., Doesburg, K., Iwasaki, T., & Mierau, I. (1999). Screening of lactic acid bacteria for bile salt hydrolase activity. *Journal of Dairy Science*, *82*(12), 2530–2535. doi: 10.3168/jds.S0022-0302(99)75506-2.

Therdtatha, P., Tandumrongpong, C., Pilasombut, K., Matsusaki, H., Keawsompong, S., & Nitisinprasert, S. (2016). Characterization of antimicrobial substance from *Lactobacillus salivarius* KL-D4 and its application as biopreservative for creamy filling. *Springerplus*, *5*(1), 1060. doi: 10.1186/s40064-016-2693-4.

Todorov, S. D. (2009). Bacteriocins from *Lactobacillus plantarum* – production, genetic organization and mode of action: produção, organização genética e modo de ação. *Brazilian Journal of Microbiology*, *40*(2), 209–221. doi: 10.1590/S1517-83822009000200001.

Tok, E., & Aslim, B. (2010). Cholesterol removal by some lactic acid bacteria that can be used as probiotic. *Microbiology and Immunology*, *54*(5), 257–264. doi: 10.1111/j.1348-0421.2010.00219.x.

Torres, N. I., Sutyak Noll, K., Xu, S., Li, J., Huang, Q., Sinko, P. J., et al. (2013). Erratum to: Safety, formulation and in vitro antiviral activity of the antimicrobial peptide subtilosin against herpes simplex virus type 1. *Probiotics and Antimicrobial Proteins*, *5*(1), 36. doi: 10.1007/s12602-013-9128-0.

Trabelsi, I., Slima, S. B., Chaabane, H., & Riadh, B. S. (2015). Purification and characterization of a novel exopolysaccharides produced by *Lactobacillus* sp. Ca6. *International Journal of Biological Macromolecules*, *74*, 541–546. doi: 10.1016/j.ijbiomac.2014.12.045.

Tukenmez, U., Aktas, B., Aslim, B., & Yavuz, S. (2019). The relationship between the structural characteristics of *Lactobacilli*-EPS and its ability to induce apoptosis in colon cancer cells in vitro. *Scientific Reports*, *9*(1), 8268. doi: 10.1038/s41598-019-44753-8.

van Kranenburg, R., Marugg, J. D., van Swam, I. I., Willem, N. J., & de Vos, W. M. (1997). Molecular characterization of the plasmid-encoded eps gene cluster essential for exopolysaccharide biosynthesis in *Lactococcus lactis*. *Molecular Microbiology*, *24*(2), 387–397. doi: 10.1046/j.1365-2958.1997.3521720.x.

Venkatesh, P., Balraj, M., Ayyanna, R., Ankaiah, D., & Arul, V. (2016). Physicochemical and biosorption properties of novel exopolysaccharide produced by *Enterococcus faecalis*. *LWT—Food Science and Technology*, *68*, 606–614. doi: 10.1016/j.lwt.2016.01.005.

Vidhyalakshmi, R., Valli, N. C., Narendra Kumar, G., & Sunkar, S. (2016). *Bacillus circulans* exopolysaccharide: Production, characterization and bioactivities. *International Journal of Biological Macromolecules*, *87*, 405–414. doi: 10.1016/j.ijbiomac.2016.02.001.

Vieco-Saiz, N., Belguesmia, Y., Raspoet, R., Auclair, E., Gancel, F., Kempf, I., et al. (2019). Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. *Frontiers in Microbiology*, *10*(57), 1–17. doi: 10.3389/fmicb.2019.0005730804896.

Wachi, S., Kanmani, P., Tomosada, Y., Kobayashi, H., Yuri, T., Egusa, S., et al. (2014). *Lactobacillus delbrueckii* TUA4408L and its extracellular polysaccharides attenuate enterotoxigenic *Escherichia coli*-induced inflammatory response in porcine intestinal epitheliocytes via Toll-like receptor-2 and 4. *Molecular Nutrition & Food Research*, *58*(10), 2080–2093. doi: 10.1002/mnfr.201400218.

Walter, J., Schwab, C., Loach, D. M., Ganzle, M. G., & Tannock, G. W. (2008). Glucosyltransferase A (GtfA) and inulosucrase (Inu) of *Lactobacillus reuteri* TMW1.106 contribute to cell aggregation, in vitro biofilm formation, and colonization of the mouse gastrointestinal tract. *Microbiology*, *154*(Pt 1), 72–80. doi: 10.1099/mic.0.2007/010637-0.

Wang, G., Manns, D. C., Churey, J. J., & Worobo, R. W. (2014). Development of a homologous expression system for and systematic site-directed mutagenesis analysis of thurincin H, a bacteriocin produced by *Bacillus thuringiensis* SF361. *Applied and Environmental Microbiology*, *80*(12), 3576–3584. doi: 10.1128/AEM.00433-14.

Wang, J., Zhao, X., Yang, Y., Zhao, A., & Yang, Z. (2015). Characterization and bioactivities of an exopolysaccharide produced by *Lactobacillus plantarum* YW32. *International Journal of Biological Macromolecules*, *74*, 119–126. doi: 10.1016/j.ijbiomac.2014.12.006.

Wang, L., Wang, Y., Li, Q., Tian, K., Xu, L., Liu, G., et al. (2019). Exopolysaccharide, isolated from a novel strain *Bifidobacterium breve* lw01 possess an anticancer effect on head and neck cancer – Genetic and biochemical evidences. *Frontiers in Microbiology*, *10*(1044), doi: 10.3389/fmicb.2019.01044.

Wang, Y., Li, C., Liu, P., Ahmed, Z., Xiao, P., & Bai, X. (2010). Physical characterization of exopolysaccharide produced by *Lactobacillus plantarum* KF5 isolated from Tibet Kefir. *Carbohydrate Polymers*, *82*(3), 895–903. doi: 10.1016/j.carbpol.2010.06.013.

Yang, S. C., Lin, C. H., Sung, C. T., & Fang, J. Y. (2014). Antibacterial activities of bacteriocins: Application in foods and pharmaceuticals. *Frontiers in Microbiology*, *5*, 241. doi: 10.3389/fmicb.2014.00241.

Yilmaz, M. T., Dertli, E., Toker, O. S., Tatlisu, N. B., Sagdic, O., & Arici, M. (2015). Effect of in situ exopolysaccharide production on physicochemical, rheological, sensory, and microstructural properties of the yogurt drink ayran: An optimization study based on fermentation kinetics. *Journal Dairy Science*, *98*(3), 1604–1624. doi: 10.3168/jds.2014-8936.

Zacharof, M. P., & Lovitt, R. W. (2012). Bacteriocins produced by lactic acid bacteria a review article. *APCBEE Procedia*, *2*, 50–56. doi: 10.1016/j.apcbee.2012.06.010.

Zannini, E., Waters, D. M., Coffey, A., & Arendt, E. K. (2016). Production, properties, and industrial food application of lactic acid bacteria-derived exopolysaccharides. *Applied Microbiology and Biotechnology*, *100*(3), 1121–1135. doi: 10.1007/s00253-015-7172-2.

Zhang, L., Liu, C., Li, D., Zhao, Y., Zhang, X., Zeng, X., et al. (2013). Antioxidant activity of an exopolysaccharide isolated from *Lactobacillus plantarum* C88. *International Journal of Biological Macromolecules*, *54*, 270–275. doi: 10.1016/j.ijbiomac.2012.12.037.

Zheng, G., Yan, L. Z., Vederas, J. C., & Zuber, P. (1999). Genes of the sbo-alb locus of *Bacillus subtilis* are required for production of the antilisterial bacteriocin subtilosin. *Journal of Bacteriology*, *181*(23), 7346–7355.

Zhou, K., Zeng, Y., Yang, M., Chen, S., He, L., Ao, X., et al. (2016). Production, purification and structural study of an exopolysaccharide from *Lactobacillus plantarum* BC-25. *Carbohydrate Polymers*, *144*, 205–214. doi: 10.1016/j.carbpol.2016.02.067.

Zhu, Y., Wang, C., Jia, S., Wang, B., Zhou, K., Chen, S., et al. (2018). Purification, characterization and antioxidant activity of the exopolysaccharide from *Weissella cibaria* SJ14 isolated from Sichuan paocai. *International Journal of Biological Macromolecules*, *115*, 820–828. doi: 10.1016/j.ijbiomac.2018.04.067.

Zouhir, A., Hammami, R., Fliss, I., & Hamida, J. B. (2010). A new structure-based classification of Gram-positive bacteriocins. *The Protein Journal*, *29*(6), 432–439. doi: 10.1007/s10930-010-9270-4.

Chapter 20

Probiotics in Shrimp Aquaculture

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1 Introduction

The word probiotics originates from the Greek word "pro" and "bios," which means "for life" and is currently used to name bacteria associated with beneficial effects for human and animals. The terms probiotic bacteria used synonymously probiotics, probiont, beneficial bacteria, or friendly bacteria (Rao, 2002). Probiotics means inhibit harmful bacteria, promote good digestion, and increase immune function and resistance to infection. The major role of probiotics is resistance to infectious diseases and producing antibacterial substance with the purpose of inhibiting the growth of bacterial pathogen (Ringo et al., 2007). Probiotics are used in aquaculture to modify and manipulate microbial population and increase the growth rate of fishes (Lilly & Stillwell, 1965). They are bio-friendly agent like lactic acid bacteria and *Bacillus* sp. and can be introduced into the culture environment to control with pathogenic bacteria. The probiotics are *Lactobacillus* sp., *Streptococcus* sp., *Cytophaga* sp., *Pseudomonas* sp., *Alteromonas* sp., *Aeromonas* sp., and *Enterococcus* sp.

2 Definition

The term "probiotic" was first introduced by Parker (1974) defined as "organisms and substances which contribute to intestinal microbial balance."

According to FAO/WHO (2001), probiotic was redefined as "live microorganisms which, when administrated in adequate amounts, confer a health benefit on the host."

Probiotics are defined as live organisms that contribute to intestinal microbial balance.

Coeuret, Guwguen, and Vernoux (2004) defined it as "living microorganisms that, upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition."

"A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" (Fuller, 1989).

Lara-Flores and Aguirre-Guzman (2009) denote probiotic as a live microbial food supplement that confers health benefits or disease resistance to the host.

Probiotics, defined as beneficial live microorganisms when administered to a host at an effective dose, provide a potential alternative strategy for reducing disease outbreaks.

Probiotics are defined as "living microbial cells administered as dietary supplements with the aim of improving health" (Tannock, 1997).

3 Types of probiotics

Based on their mode of application, there are two types of probiotics:

1. Feed probiotics
2. Pond probiotics

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00020-4
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1. **Feed probiotics**

   Feed probiotics are those that are applied through the feed. Probiotics can be incorporated in the diet in two ways:
   a. Through artificially produced (dry) feed including pellets, pulverize, granules, flakes, or microencapsulated diets.
   b. Natural live feed organisms that are nurtured in probiotic-enriched media results in the encapsulation of the probiotic organisms in a process called bioencapsulation (Nayak, Reddy, & Pawar, 2003).

2. **Pond probiotics**

Health of aquatic organisms deteriorates rapidly with increasing environmental stress mainly in the form of low dissolved oxygen (DO), accumulation of dissolved ammonia, nitrite anoxic conditions, and hydrogen sulfide in the sediments. Several microbial supplements can be used to prevent their establishment in ponds. Pond probiotics remit on the bottom sediments and act from there. Some important actions of pond probiotics are biocontrol and bioremediation.

4 Microorganisms of probiotic

Aquaculture probiotic microorganisms are *Lactobacillus*, *Enterococcus*, *Bacillus*, *Aeromonas*, *Alteromonas*, *Arthrobacter*, *Bifidobacterium*, *Clostridium*, *Lactobacillus*, *Microbacterium*, *Paenibacillus*, *Pediococcus*, *Phaeobacter*, *Pseudoalteromonas*, *Pseudomonas*, *Rhodosporidium*, *Roseobacter*, *Saccharomyces*, *Streptomyces*, and *Vibrio*. The beneficial immune effects of probiotics are as well accepted in finfish. In recent studies, native probiotic bacteria were isolated and fed back to their hosts (Moriarty, 1998). Probiotics are used as curative, preventive, growth supplements and functional food for human health (Pandiyan et al., 2013). Gram-positive and Gram-negative bacteria and many other organisms like yeast, bacteriophages, and algae (Mukherjee et al., 2016; Pandiyan et al., 2013). Lactic acid bacteria have been widely used as probiotic strains that exist in the intestine of healthy fishes such as the *Lactobacilli* and *Bifidobacteria*. Some Gram-positive bacteria as *Bacillus*, *Enterococcus*, *Streptococcus* act as usual probiotic strains that are the main gastrointestinal microbiota (Prasad, Baghel, & Kumar, 2003). On the other hand, Gram-negative facultative anaerobes are dominant in gastrointestinal tract (GIT) of fishes as well as symbiotic anaerobes triumph in the behind part of digestive tract of tropical herbivorous fishes (Pandiyan et al., 2013).

5 Probiotics in aquaculture

5.1 Mechanisms of probiotics

Probiotics improve the feed efficiency and control microbiota including (1) competition with other species for binding (colonization) sites; (2) contributing to nutrient availability and improve feed digestibility and feed utilization by enzymatic contribution; (3) bacteria-mediated direct uptake of dissolved organic material; (4) improve immune response against infectious pathogens; and (5) antiviral effects (Balcazar et al., 2006; Kesarcodi-Watson, Kaspar, Lategan, & Gibson, 2008).

Biocontrol is a process in which they eliminate the pathogenic biota through antagonistic properties, by competition and species diversity. Probiotic bacteria may stabilize the intestinal microbiota and bacterial antagonism applied to the preparation of new probiotics. Some of the antagonistic bacteria include *Bacillus* spores, *Roseobacter* sp., *Carnobacterium divergens*, *Pseudomonas* sp., *Alteromonas*, and *Pseudoalteromonas* against pathogens like *Vibrio harveyi*, *Vibrio anguillarum*, *Edwardsiella*, *Aeromonas*, and *Pasteurella*.

Probiotics in aquaculture are used to initiate live cells of probiotics to the host animal gut to demonstrate a balanced gastrointestinal microbial flora and to increase digestive function or immune system. Probiotics, including bacterial strains, yeast, and extracted material, are supplied by this application. Probiotics are various and usually obtained from the intestines of the host animals (Sun, Yang, Ma, Song, & Li, 2012; Chantharasophon, Warong, Mapatsa, & Leelavatcharamas, 2011; Chu, Lu, Zhu, & Kang, 2011), cultured in various environments (56), and come have been evolved into commercial products which are used (Mondal, Abraham, & Babu, 2008; Fernandez, Sridhar, & Sridhar, 2011). Some probiotics that have been augmented in animal feed include bacterial species, such as *Lactobacillus* spp., *Enterococcus faecium*, *Bifidobacterium thermophilum*, *Streptomyces* spp., *Micrococcus* spp., *Pseudomonas fluorescens*, and yeast *Saccharomyces cerevisiae*.

Furthermore, probiotics have been out into culture ponds to improve water quality and the residence of cultured animals (Moriarty, 1998). The effect of probiotics can be described by bioaugmentation or biocontrol mechanisms by which the microbial ecology of the water and soil quality are improved (Rengpipat, 2005).

5.2 Competitive exclusion

Probiotics can restrain to colonic cell lines and mucin, which is conceptual to aid colonization of the animal gut system (Ouwehand, Tolkko, Kulmala, Salminen, & Salminen, 2000; Juntunen, Kirjavainen, Ouwehand, Salminen, & Isolauri, 2001). The microflora of the GIT of aquatic animals can be altered by ingestion of other microorganisms. However, the dominant bacteria in fish intestine are quite different from those see in mammals (Gatesoupe, 2005). Bacterial antagonism is a common occurrence in nature. Probiotics may avert opportunistic pathogens from colonizing the surface of eggs or the GIT by producing antimicrobial compounds or by outcompeting them for nutrients (Bandyopadhyay & Mohapatra, 2009). Certain pathogens produce proteolytic enzymes that can break down and digest the bacteria that approaches them, the most unusual being *Streptococcus* and *Bacillus pyocyaneus*. *Bacillus subtilis* BT23 has repressive effects against vibriosis in tiger shrimp *Penaeus monodon* (Vaseeharan & Ramasam, 2003).

5.3 Nutrient and enzymatic contributions to digestion

Agrobacterium sp., *Brevibacterium* sp., *Clostridium* sp., *Microbacterium* sp., *Pseudomonas* sp., and *Staphylococcus* sp. can supplement the host's nutrition, mainly by supplying fatty acids and vitamins to host cells (Sakata, 1990; Ringo, Strom, & Tabachek, 1995). Some fish gut microbiota may engage directly in the digestion processes. There are enzyme-producing microbiota such as *Bacillus* and Enterobacteriaceae (*Acinetobacter* sp., *Aeromonas* sp., *Flavobacterium* sp., *Photobacterium* sp., *Pseudomonas* sp., *Vibrio* sp., *Microbacterium* sp., *Micrococcus* sp., *Staphylococcus* sp.), and some unidentified anaerobes and yeasts are potential reporters.

6 Immune system promoters

Probiotics were presumed to play an important role in the stimulation of the immune response in shrimp (Lakshmi & Sai Gopal, 2013). The use of *Bacillus* sp. (strain S11) gives disease protection by activating both cellular and humoral immune defenses in tiger shrimp (*P. monodon*). The administration of a mixture of bacterial strains (*Bacillus* spp. and *Vibrio* spp.) positively influenced the growth and survival of white shrimp juveniles and grants a protective effect against pathogens *V. harveyi* and white spot syndrome virus. This protection was assign to the stimulation of the immune system by increasing phagocytosis and antibacterial activity (Chu et al., 2011). *Bacillus* spp. was used to control the *Vibrio* spp. infection to penaeid shrimp (Moriarty, 1998).

7 Water quality improvement

The susceptibility of cultured aquatic species to concentrations of high nitrogenous compounds is ammonia, nitrite, and nitrate, species-specific but these compounds are high, which affect animals in aquaculture and cause high mortality. The effectiveness of *Bacillus* spp., for improving water quality by affecting the composition and exuberance of waterborne microbes associated with farmed species, was assessed (Dalmin, Kathiresan, & Purushothaman, 2001). *Bacillus* spp. were associated with improvement of water quality, reduction of pathogenic vibrios, enhancement of survival and growth rate, and improved health status of juvenile *P. monodon* (Dalmin et al., 2001; Ngan & Phu, 2011). Other beneficial bacterial species are *Nitrobacter*, *Pseudomonas*, *Enterobacter*, *Cellulomonas*, and *Rhodopseudomonas*.

8 Bioremediation

Nayak et al. (2003) advocated bioremediation in which they maintain water quality through reduction in biochemical and chemical oxygen demands (CODs). The responsible bacteria have the capacity to break down complex organic matter to simpler material and are also capable of oxidizing toxic elements (ammonia, nitrite) and provide into harmless. Bioremediation products' results are cleaner pond bottoms:

- Reduced blue-green algae loads (via competition for nutrients-these products are not algaecides).
- Reduced green *vibrio* counts (on TCBS, the *vibrio* produces green colonies).
- Reduced ammonia levels (broken down by the bacteria).
- Reduced disease outbreaks (a result of a change in the microbial flora).
- Good feed efficiency (a result of a cleaner environment)
- Healthier shrimp

Information on the efficiently of water probiotic on water quality parameters of shrimp culture ponds is not adequate, and the purpose to the present study was conducted to observe the effect of water probiotic, on the physicochemical parameters of the both control and experimental ponds of *Litopenaeus vannamei.*

9 Materials and methods

The present study was undertaken at a shrimp farms in Thirupoondi-Nagapattinam district, India. The study was conducted in tank culture method.

10 Probiotic feed preparation

Probiotic supplemented feed prepared as followed by standard method (Plate 20.1). The 24-h-old bacterial culture was maintained in the nutrient broth, and the bacterial species were harvested by centrifuging at 10,000 rpm for 10 min. These harvested bacteria were washed thrice with phosphate buffer saline (PBS, pH 7.4), and the bacterial cells were resuspended in PBS. These resuspended bacteria were mixed uniformly to the feed pellets by using sprayer. The prepared probiotic-blended feed was then dried at 40°C and packed in air tight polythene bags and stored in 4°C. Test diets PB1 supplemented with *Bacillus coagulans* at 10 billion CFU/kg and *Lactobacillus acidophilus* at 8 billion CFU/kg feed and *B. coagulans* and *L. acidophilus* test diets were prepared once in every 15 days.

10.1 Tank culture experiments

According to Ballester, Wasielesky, Cavalli, and Abreu (2007), Plate 20.2, 12 blue Gallon plastic tanks (543-cm diameter and 717-cm deep) were used to test 4 treatments (triplicates):

Group 1 (G1), as a control tank without probiotics,
Group 2 (G2), with *B. coagulans* + commercial feed treated,
Group 3 (G3) with *L. acidophilus* + commercial feed treated, and
Group 4 (G4) with mixed *B. coagulans* + *L. acidophilus* + commercial feed, respectively.

The microbial consortium was prepared in feed + *B. coagulans* and *L. acidophilus* probiotics in the ratio 3:1. Each tank was filled with water and conditioned parameters. Twenty eight day old *L. vannamei* postlarvae (PL28) purchased from a



PLATE 20.1 Preparation of microbial consortium for trial plot of shrimp aquaculture.



PLATE 20.2 Application of feed in the tank.

local commercial hatchery in Nagapattinam district were stocked at a density of 450/tank (510 PL/m^2) on June 12, 2018, and the shrimps were cultured for 90 days to September 10, 2018. Commercial shrimp feed (HaimaTM) was procured from local market in Pattukkottai and provided feed (microbial consortium + probiotics) in two times daily. The amount of feed was adjusted daily for each tank based upon the amount of uneaten food observed. Every 40 days interval of physicochemical parameters in water, survival rate (SR), morphometric parameters, and biochemical parameters were performed. Water quality parameters for every 40th day (40, 80, and 120 days).

11 Assessment of physicochemical parameters

The following parameters were tested: color, temperature, pH, turbidity (TD), salinity, alkalinity, DO, biochemical oxygen demand (BOD), COD, calcium, nitrate, nitrite, iron, and ammonia were analyzed. The parameters were analyzed according to the Standard Methods for the Examination of Water and Wastewater (Apha, 1992). To observe spatial distribution of shrimp in water, pictures were taken from all treatments twice daily (10:00 a.m. and 4:00 p.m.) by a digital camera (Canon IXUS 70) that was fixed to a steel bar above tanks during the days of culture.

12 Assessment of growth performance

Growth parameters were recorded on once in 15 days of culture. *L. vannamei* were randomly collected from grow-out tank in the forenoon (10:00 a.m.) using cast net. However, sampling was avoided during molting period and during cloudy and rainy conditions for reducing experimental errors. The variations in net weight gain were calculated by the method of Sambhu and Jayaprakas (2001), measuring the length (Plate 20.3) and weight (Plate 20.4) followed by estimation of SR, specific growth rate (SGR) determined by the method of Ravi, Ajmalkhan, and Rajagopal (1998) and average daily growth (ADG) (Mustafa & Ridzwan, 2000) using the following formula:

Weight gain (%) = Final weight (g) − Initial weight (g)/Initial weight (mg).
Length gain (%) = Final length (mm) − Initial length (mm)/Initial length (mm) × 100.
SGR (%) = (In final weight − In initial weight)/Days of culture × 100.
ADG (g/day) = Final weight (mg) − Initial weight (mg)/Experimental duration (days).
Survival (%) = Shrimp no. at the end of experiment/Shrimp no. at the beginning of experiment × 100.

In every interval, the SR was measured in percentage, length (mm) use in scale, weight (g) in balance, and feed conservation rate were recorded. After that the biochemical parameters of carbohydrate, lipid, protein, calcium, phosphorus, potassium, magnesium, sodium, manganese, vitamin A, vitamin C, and vitamin E were analyzed using standard procedures.

13 Results and discussion

The maintenance of good water quality is essential for optimum growth and survival of shrimps. Good water quality is characterized by adequate DO, temperature, pH, and salinity. All the water quality parameters in the three experimental tanks with control were found within suitable ranges for *L. vannamei* culture throughout the experimental period.

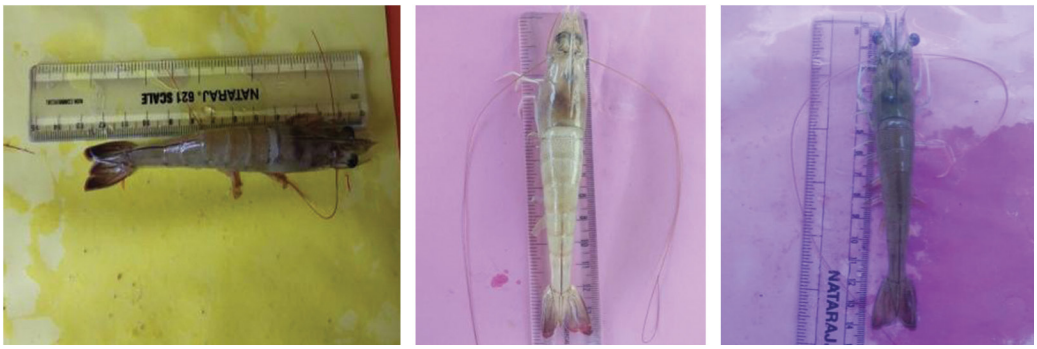


PLATE 20.3 Length of shrimp. Morphometric length measurement of *L. vannamei.*



PLATE 20.4 Weight of shrimp. Average body weight after treatment of *L. vannamei.*

The current analysis stated the efficacy of probiotic supplements on the *L. vannamei*, and their physicochemical parameters were investigated. The probiotic consortium of *B. coagulans* and *L. acidophilus* displayed significant role than that of the individual application, due to the combined effect of probiotic bacteria has exhibited through the physicochemical parameters observations of 40, 80, and 120 days of culturing tank condition, respectively. However, the physicochemical parameters have main recognizing factors for the growth and development of shrimp aquaculture industry.

The physical parameters of water play crucial role in the culture systems. Maintenance of water quality is essential for optimum growth and survival of shrimp. Excess feed, fecal matter, and metabolites will exert tremendous influence on the water quality of shrimp farm (Soundarapandian & Gunalan, 2008; Gunalan, Soundarapandian, & Dinakaran, 2010).

14 Determination of physicochemical parameters of probiotics supplemented shrimp aquaculture tank with various days of incubation

The physicochemical parameters like color, temperature, pH, TD, salinity, alkalinity, DO, BOD, COD, calcium, nitrate, nitrite, iron, and ammonia was slight color with transparent 10.1°C, 7.58, 24.1 TD, 30.5 ppt, 120, 7.5, 1.20, 14.2, 12.8, 0.04, 0.9, 0.002, and 0.01 mg/L recorded in the introducing *B. coagulans* in the shrimp aquaculture, respectively, whereas *L. acidophilus* effect as a probiotics supplemented tank (after 40 days) of physiochemicals like color, temperature, pH, TD, salinity, alkalinity, DO, BOD, COD, calcium, nitrate, nitrite, iron, and ammonia was slight greenish color with transparent 10.3°C, 7.50, 37.2 TD, 31.2 ppt, 136, 7.0, 1.30, 12.3, 19.5, 0.06, 0.10, 0.024, and 0.020 mg/L observed from the results, respectively. The combination of *B. coagulans* and *L. acidophilus* efficacy shrimp aquaculture tank after 40 days intervals were performed. In the physicochemical parameters like color, temperature, pH, TD, salinity, alkalinity, DO, BOD, COD, calcium, nitrate, nitrite, iron and ammonia was slight greenish color with transparent 10.2°C, 7.48, 32 TD, 30.7 ppt, 144, 8.3, 1.50, 15.7, 14.2, 0.02, 0.08, 0.17 and 0.01 mg/L represented when compared with control (untreated), respectively (Table 20.1).

TABLE 20.1 Effect of probiotic supplements on the *Litopenaeus vannamei* and analysis of physicochemical parameters of cultured shrimp aquaculture tank (40 days).

S. no.	Name of the parameters	Control	*Bacillus coagulans*	*Lactobacillus acidophilus*	*B. coagulans* and *L. acidophilus*
1		Color		Slight greenish color with transparent	
2	Temperature (°C)	10.3	10.1	10.3	10.2
3	pH	7.40	7.58	7.50	7.48
4	TD	25.5	24.1	37.2	32
5	Salinity (ppt)	26.3	30.5	31.2	30.7
6	Alkalinity (mg/L)	136	120	136	144
7	DO (mg/L)	7.9	7.5	7.0	8.3
8	BOD (mg/L)	1.04	1.20	1.30	1.50
9	COD (mg/L)	14.4	14.2	12.3	15.7
10	Calcium (mg/L)	16.3	12.8	19.5	14.2
11	Nitrate (mg/L)	0.02	0.04	0.06	0.02
12	Nitrite (mg/L)	0.04	0.9	0.10	0.08
13	Iron (mg/L)	0.02	0.022	0.024	0.17
14	Ammonia (mg/L)	0.01	0.01	0.020	0.01

BOD, Biochemical oxygen demand; *COD*, chemical oxygen demand; *DO*, dissolved oxygen; *TD*, turbidity.

The estimation of physicochemical parameters like color, temperature, pH, TD, salinity, alkalinity, DO, BOD, COD, calcium, nitrate, nitrite, iron and ammonia were slight greenish color with transparent 30.2°C, 7.68, 48.1 TD, 30.5 ppt, 162, 8.5, 2.50, 28.6, 26.8, 0.07, 0.10, 0.025, and 0.02 mg/L recorded in the *B. coagulans* probiotic supplemented shrimp aquaculture tank after 80 days, respectively. Whereas *L. acidophilus* probiotic bacteria supplemented physicochemical parameters like color, temperature, pH, TD, salinity, alkalinity, DO, BOD, COD, calcium, nitrate, nitrite, iron, and ammonia was slight greenish color with transparent, 30.4°C, 7.75, 48.3 TD, 31.2 ppt, 176, 7.9, 2.60, 29.3, 27.3, 0.08, 0.12, 0.027, and 0.023 mg/L results, respectively. The combination of both probiotic bacterial effect of *B. coagulans* and *L. acidophilus* was extraordinary results of color, temperature, pH, TD, salinity, alkalinity, DO, BOD, COD, calcium, nitrate, nitrite, iron, and ammonia with the parameters slight greenish color with transparent, 30.1°C, 7.65, 49 TD, 30.7 ppt, 167, 9.3, 1.90, 26.3, 23.5, 0.03, 0.090, 0.19, and 0.01 mg/L recorded and significant results observed, respectively (Table 20.2).

The investigation of physicochemical parameters of probiotic supplemented shrimp aquaculture tank after 120 days were analyzed. The physicochemical parameters like color, temperature, pH, TD, salinity, alkalinity, DO, BOD, COD, calcium, nitrate, nitrite, iron, and ammonia was transparent, 31.4°C, 8.15, 44.3 TD, 32.3 ppt, 199, 4.9, 3.41, 31, 41, 0.01, 0.027, 0.027, and 0.027 mg/L observed after the treated *B. coagulans* after 120 days, respectively. To assess the study of *L. acidophilus* probiotic supplement physicochemical parameters like color, temperature, pH, TD, salinity, alkalinity, DO, BOD, COD, calcium, nitrate, nitrite, iron, and ammonia was water, 30.9°C, 8.20, 42.6 TD, 32.4 ppt, 208, 4.3, 3.90, 34, 39, 0.13, 0.033, 0.039, and 0.031 mg/L recorded with specific incubation, respectively. In the case of combined effect of probiotic bacteria more and more yield production was color, temperature, pH, TD, salinity, alkalinity, DO, BOD, COD, calcium, nitrate, nitrite, iron, and ammonia with physicochemical parameters analyzed. However, the combined effect of probiotic bacteria has excellent changes in the shrimp aquaculture technology (Table 20.3).

The water quality parameters during days of culture were within acceptable for growth and survival of normal shrimp. Although pond conditions used in this study were slightly different such as pond size, stocking density, water depth, and cultured period, water quality parameters were similar each other such as DO, pH, salinity, and temperature. The water transparency during cultured periods was range 10–20 cm. The application of zero water exchange and intensive feeding rates so population of phytoplankton and bacteria in the water column increase and water color was also brown in color observed. DO profiles all day cultures were good enough, because it has been used intensively aeration 24 h all culture periods by using paddle wheels. Photosynthesis, nitrification, and heterotrophic processes that likely to occur in control system possibly resulted in pH fluctuation because of alteration of CO_2 concentration and pH changed in water (Ebeling, Timmons, & Bisogni, 2006). Although water quality indicators such as total ammonia nitrogen (TAN), nitrite, nitrate,

TABLE 20.2 Effect of probiotic supplements on the *Litopenaeus vannamei* and analysis of physicochemical parameters of cultured shrimp aquaculture tank (80 days).

S. no.	Name of the parameters	Control	*Bacillus coagulans*	*Lactobacillus acidophilus*	*B. coagulans* and *L. acidophilus*
1		Color		Slight greenish color with transparent	
2	Temperature (°C)	10.3	10.1	10.3	10.2
3	pH	7.40	7.58	7.50	7.48
4	TD	25.5	24.1	37.2	32
5	Salinity (ppt)	26.3	30.5	31.2	30.7
6	Alkalinity (mg/L)	136	120	136	144
7	DO (mg/L)	7.9	7.5	7.0	8.3
8	BOD (mg/L)	1.04	1.20	1.30	1.50
9	COD (mg/L)	14.4	14.2	12.3	15.7
10	Calcium (mg/L)	16.3	12.8	19.5	14.2
11	Nitrate (mg/L)	0.02	0.04	0.06	0.02
12	Nitrite (mg/L)	0.04	0.9	0.10	0.08
13	Iron (mg/L)	0.02	0.022	0.024	0.17
14	Ammonia (mg/L)	0.01	0.01	0.020	0.01

BOD, Biochemical oxygen demand; *COD*, chemical oxygen demand; *DO*, dissolved oxygen; *TD*, turbidity.

TABLE 20.3 Effect of probiotic supplements on the *Litopenaeus vannamei* and analysis of physicochemical parameters of cultured shrimp aquaculture tank (120 days).

S. no.	Name of the parameters	Control	*Bacillus coagulans*	*Lactobacillus acidophilus*	*B. coagulans* and *L. acidophilus*
1		Color		Greenish color with transparent	
2	Temperature (°C)	31	31.4	30.9	30.3
3	pH	8.0	8.15	8.20	7.95
4	TD	43	44.3	42.6	44.0
5	Salinity (ppt)	313	32.3	32.4	31.50
6	Alkalinity (mg/L)	178	199	208	185
7	DO (mg/L)	5.2	4.9	4.3	7.6
8	BOD (mg/L)	3.02	3.41	3.90	2.60
9	COD (mg/L)	29	31	34	30
10	Calcium (mg/L)	32	41	39	41
11	Nitrate (mg/L)	0.06	0.01	0.13	0.048
12	Nitrite (mg/L)	0.025	0.027	0.033	0.016
13	Iron (mg/L)	0.015	0.027	0.039	0.024
14	Ammonia (mg/L)	0.015	0.027	0.031	0.027

BOD, Biochemical oxygen demand; *COD*, chemical oxygen demand; *DO*, dissolved oxygen; *TD*, turbidity.

and alkalinity were not monitored during this experiment, the other water qualities such as water transparency, DO, pH, salinity, and temperature were suitable for normal shrimp growth also reported (Kuhn, Boardman, Marsh, Lawrence, & Flick, 2009a). Nitrite is the intermediate compound in the bacterial nitrification of ammonia into nitrate (in oxidizing environments) or the production of nitrate denitrification (in reducing environments) and is toxic to aquatic organisms, causing mortality in culture systems (Brownell, 1980; Tsai & Chen, 2002) *L. vannamei*, is widely cultured in Central and South

America (Wen-Young, 1998) and tolerates the salinities of 2–45 ppt (Samocha, Lawrence, & Pooser, 1998). Muthu (1980), Soundarapandian and Gunalan (2008), and Karthikeyan (1994) reported a salinity range of 10–35 ppt was important for *P. monodon* culture. Our results favor the growth of *L. vannamei*.

During the experimental period, the temperature was recorded between 32°C and 35°C (Allan & Maguire, 1992). pH is one of the important environmental parameters that decide physiological process of shrimps. The optimum range of pH 6.8–8.7 should be maintained for maximum growth and production (Ramanathan, Padmavathy, Francis, Athithian, & Selvaranjitham, 2005). The pH of pond water is influenced by many factors, including pH of source water, acidity of bottom soil, and shrimp culture inputs and biological activity, according to our studies similar to (Wang et al., 2004) the recommended range of pH for *L. vannamei* culture.

DO affects the solubility and availability of many nutrients. Low levels of DO can cause damages in oxidation state of substances from the oxidized to reduced form. In the present study, DO was found 4.3–9.3 mg/L in probiotics and control ponds, respectively. Low levels of dissolve oxygen tension hampers metabolic performances in shrimp and can be reduce growth and molting and cause mortality (Gilles, 2001). Soundarapandian, Ramanan, and Dinakaran (2010) found 3.2–4.2 ppm DO.

Ammonia is the principal and end product of protein catabolism of organisms, and it is excreted through gills. It is also formed by decay of organic matter. Under anaerobic condition, sulfate is also reduced to ammonia. In the present study, TAN was 0.01–0.23 in probiotics treated and control ponds, respectively. Ammonia level should be less than 1 ppm (Soundarapandian et al., 2010). The preset results were similar to the findings of earlier workers (Hossain, Kamal, Mannan, Bhuyian, & Hossain, 2013; Soundarapandian et al., 2010). Thus, maintaining the ammonia level probiotics helps in maintaining good water quality and there by keeps the shrimps disease free.

The growth and survival of *L. vannamei* in brackish water ponds of 10–35 ppt were ideal for shrimp culture. However, the shrimp tolerates the salinity even 2.45 ppt. Wang et al. (2004) reported the favorable pH from 7 to 8.6 for *L. vannamei*, while it was observed at a pH of 7 in the study of Fast and Lannan (1992); best shrimp growth was observed in a temperature range from 24°C to 32°C.

Intensive aquaculture systems produced a large number of wastes because of highly commercial feed utilization in this system, so that it was rapidly accumulated organic and inorganic nitrogen materials which reduce water quality most this system. Application of probiotic bacteria was carried out every week to control water quality that added in commercial feed and water treatment. The use of bioaugmentation agent was highly beneficial in maintain water quality shrimp growth due to high bacterial population in this system will degrade the waste products without depleting the nutrients need for phytoplankton growth.

Effect of *B. coagulans* on the morphometric analysis of *L. vannamei* culture like length, weight, weight gain, survival, FCR was 11.3 mm, 112, 6.3 g, 75%, and 1:1.2. In *L. acidophilus* on the morphometric analysis of length, weight, weight gain, survival, FCR with 10.31 mm, 123, 5.4 g, 70.60% and 1:1.2 recorded, respectively. The *B. coagulans* and *L. acidophilus* culture tank parameters like length, weight, weight gain, survival, and FCR with 12.3 mm, 130, 5.6 g, 82.50%, and 1:1.2 recorded 40 days of growth condition.

Whereas 80 days of *L. vannamei*, has moderate growth was *B. coagulans* 13.5 mm, 154, 8.6 g, 90% and 1:1.35 with parameters length, weight, weight gain, survival, FCR represented from the study, respectively, in the *L. acidophilus*, parameters like length, weight, weight gain, survival, and FCR with 13.3 mm, 145, 6.8 g, 90.66% and 1:1.35 recorded, respectively. While the mixed inoculums of *B. coagulans* and *L. acidophilus* culture tank parameters of 14.2, 1.52, 6.4, 92.66, and 1:1.35 in length, weight, weight gain, survival, and FCR. In the same time, 120 days culture tank was extraordinary growth and development were measured and analyzed in *B. coagulans* parameters like length, weight, weight gain, survival, and FCR was 14.9 mm, 219, 6.5 g, 86%, and 1:1.16 observed. Then the *L. acidophilus* parameters like length, weight, weight gain, survival, and FCR was 15.3 mm, 213, 6.8 g, 85%, and 1:1.16 recorded represented. After the culture excellent growth of *B. coagulans* and *L. acidophilus*, parameters like length, weight, weight gain, survival, and FCR were 15.8 mm, 216, 6.4 g, 86.53%, and 1:1.16 were represented, respectively. These all parameters are compared to control tank (Table 20.4).

15 Biochemical analysis of *Litopenaeus vannamei* by the effect of potential bacteria in different intervals

The biochemical analysis of carbohydrate, lipid, protein, ash, moisture, calcium, phosphorus, potassium, magnesium, sodium, manganese, and iron was 1.9 ± 0.14, 5.6 ± 0.15, 121.5 ± 0.21, 73.8 ± 0.24, 1.8 ± 0.30, 155.5 ± 0.26, 16.33 ± 0.10, 63.61 ± 0.32, 57.34 ± 0.08, 6.45 ± 0.07, 0.84 ± 0.03, and 5.40 ± 0.09 mg/g recorded with *B. coagulan*s of 40 days of *L. vannamei* represented, whereas the effect *of L. acidophilus* bacteria showed that the biochemicals like 1.10 ± 0.17, 6.6 ± 0.19, 122.6 ± 0.17,

TABLE 20.4 Growth and development of characteristics and survival of *Litopenaeus vannamei* culture tank with different incubation.

S. no.	Name of the parameters	Control	*Bacillus coagulans*	*Lactobacillus acidophilus*	*B. coagulans* and *L. acidophilus*
Period of 40 days					
1.	Length (mm)	09.1	11.3	10.31	12.3
	11.3	10.31	12.3		
2.	Weight (g)	100	112	123	130
3.	Weight gain (g)	1.4	6.3	5.4	5.6
4.	Survival (%)	66	75	70.60	82.50
5.	FCR	1:1.1	1:1.2	1:1.2	1:1.2
Period of 80 days					
1.	Length (mm)	10.1	11.3	10.31	12.3
	13.5	13.3	14.2		
2.	Weight (g)	101	154	145	152
3.	Weight gain (g)	2.6	8.6	6.8	6.4
4.	Survival (%)	86	90	90.66	92.66
5.	FCR	1:1.15	1:1.16	1:1.16	1:1.16
Period of 120 days					
1.	Length (mm)	13.5	11.3	10.31	12.3
	14.9	15.3	15.8		
2.	Weight (g)	139	219	213	216
3.	Weight gain (g)	3.8	6.5	6.8	6.4
4.	Survival (%)	78	86	85	86.53
5.	FCR	1:1.15	1:1.16	1:1.16	1:1.16

FCR, Food conversion rate.

74.9 ± 1.26, 1.9 ± 0.32, 156.6 ± 0.37, 16.63 ± 0.11, 66.32 ± 0.36, 58.70 ± 0.11, 6.98 ± 0.09, 0.87 ± 0.04, and 5.68 ± 0.11 mg/g results of minimum period (40 days) incubated and analyzed with respective shrimp shell, respectively. As per combined effect of potential probiotic bacteria, *B. coagulans* and *L. acidophilus* (1:1) were more significant results of 1.15 ± 0.19, 7.6 ± 0.20, 124.6 ± 0.20, 76.9 ± 1.28, 1.9 ± 0.36, 157.7 ± 0.38, 17.63 ± 0.13, 68.34 ± 0.42, 59.71 ± 0.15, 6.99 ± 0.10, 0.89 ± 0.06, and 5.76 ± 0.14 mg/g with carbohydrate, lipid, protein, ash, moisture, calcium, phosphorus, potassium, magnesium, sodium, manganese, and iron content recorded in 40 days incubation time, respectively, compared to control, respectively (Table 20.5).

The biochemical analysis of carbohydrate, lipid, protein, ash, moisture, calcium, phosphorus, potassium, magnesium, sodium, manganese, and iron content was 1.10 ± 0.20, 6.7 ± 0.17, 123.5 ± 0.24, 77.8 ± 0.39, 1.8 ± 0.33, 156.7 ± 0.27, 17.34 ± 0.12, 66.62 ± 0.33, 58.35 ± 0.09, 7.48 ± 0.07, 0.87 ± 0.04, and 6.40 ± 0.09 mg/g recorded and treated with 80 days of *L. vannamei* represented, whereas the effect of *L. acidophilus* bacteria showed that the biochemicals and minerals 1.10 ± 0.17, 7.6 ± 0.20, 125.6 ± 0.20, 74.9 ± 1.26, 1.9 ± 0.32, 156.9 ± 0.37, 17.63 ± 0.15, 67.64 ± 0.37, 59.71 ± 0.12, 7.68 ± 0.10, 0.89 ± 0.06, and 7.68 ± 0.12 mg/g result of minimum period (80 days) incubated analyzed with respective shrimp sample (Table 20.6).

The biochemical analysis of carbohydrate lipid, protein ash, moisture, calcium, phosphorus, potassium, magnesium, sodium, manganese, and iron content was 1.13 ± 0.22, 7.8 ± 0.20, 125.6 ± 0.26, 78.56 ± 0.42, 1.13 ± 0.35, 155.9 ± 0.31, 18.65 ± 0.14, 70.73 ± 0.36, 68.8 ± 60.10, 0.88 ± 0.05, 8.40 ± 0.12 mg/g *B. coagulans* treated with 120 days of *L. vannamei* represented, whereas *L. acidophilus* treated tank showed 1.16 ± 0.18, 8.8 ± 0.24, 127.8 ± 0.28, 79.10 ± 1.29, 1.23 ± 0.36, 158.10 ± 0.39, 18.68 ± 0.17, 73.75 ± 0.38, 69.74 ± 0.15, 9.43 ± 0.10, 0.92 ± 0.08, and 8.79 ± 0.14 mg/g recorded, and the effect of mixed *B. coagulans* + *L. acidophilus* bacteria showed that the biochemicals like 1.18 ± 0.25, 9.11 ± 0.26, 129.9 ± 0.32, 82.12 ± 1.32, 1.32 ± 0.41, 161.8 ± 0.43, 19.74 ± 0.20, 76.84 ± 0.54, 73.76 ± 0.18, 9.89 ± 0.16, 0.97 ± 0.09, and 9.86 ± 0.25 mg/g result of controlled period of 120 days with incubated analyzed with respective shrimp samples (Table 20.7).

TABLE 20.5 Estimation of biochemical analysis of *Litopenaeus vannamei* culture tank for the period 40 days.

S. no.	Biochemical analysis	Quantity (mg/g) Control	*Bacillus coagulans*	*Lactobacillus acidophilus*	*B. coagulans* and *L. acidophilus*
1.	Carbohydrate	1.7 ± 0.11	1.9 ± 0.14	1.10 ± 0.17	1.15 ± 0.19
2.	Lipid	4.6 ± 0.10	5.6 ± 0.15	6.6 ± 0.19	7.6 ± 0.20
3.	Protein	109.8 ± 0.28	121.5 ± 0.21	122.6 ± 0.17	124.6 ± 0.20
4.	Ash	69.3 ± 0.22	73.8 ± 0.24	74.9 ± 1.26	76.9 ± 1.28
5.	Moisture	1.4 ± 0.05	1.8 ± 0.30	1.9 ± 0.32	1.9 ± 0.36
6.	Minerals				
	Calcium (Ca)	150.4 ± 0.16	155.5 ± 0.26	156.6 ± 0.37	157.7 ± 0.38
	Phosphorus (P)	14.51 ± 0.01	16.33 ± 0.10	16.63 ± 0.11	17.63 ± 0.13
	Potassium (K)	60.6 ± 0.13	63.61 ± 0.32	66.32 ± 0.36	68.34 ± 0.42
	Magnesium (Mg)	41.35 ± 0.04	47.34 ± 0.08	58.70 ± 0.11	62.71 ± 0.15
	Sodium (Na)	6.38 ± 0.06	6.45 ± 0.07	6.98 ± 0.09	6.99 ± 0.10
	Manganese (Mn)	0.76 ± 0.02	0.84 ± 0.03	0.87 ± 0.04	0.89 ± 0.06
	Iron	4.86 ± 0.121	5.40 ± 0.09	5.68 ± 0.11	5.76 ± 0.14

Standard deviation ± error.

TABLE 20.6 Estimation of biochemical analysis of *Litopenaeus vannamei* culture tank for the period 80 days.

S. no.	Biochemical analysis	Quantity (mg/g) Control	*Bacillus coagulans*	*Lactobacillus acidophilus*	*B. coagulans* and *L. acidophilus*
1.	Carbohydrate	1.9 ± 0.19	1.10 ± 0.20	1.10 ± 0.17	1.17 ± 0.20
2.	Lipid	5.6 ± 0.37	6.7 ± 0.17	7.6 ± 0.20	7.10 ± 0.22
3.	Protein	110.8 ± 0.30	123.5 ± 0.24	125.6 ± 0.20	127.7 ± 0.23
4.	Ash	76.3 ± 0.38	77.8 ± 0.39	74.9 ± 1.26	77.10 ± 1.29
5.	Moisture	1.6 ± 0.06	1.8 ± 0.33	1.9 ± 0.32	1.10 ± 0.38
6.	Minerals				
	Calcium (Ca)	153.7 ± 0.26	156.7 ± 0.27	156.9 ± 0.37	159.8 ± 0.39
	Phosphorus (P)	16.24 ± 0.06	17.34 ± 0.12	17.63 ± 0.15	18.65 ± 0.15
	Potassium (K)	60.59 ± 0.11	66.62 ± 0.33	67.64 ± 0.37	68.36 ± 0.45
	Magnesium (Mg)	54.69 ± 0.06	58.35 ± 0.09	59.71 ± 0.12	60.72 ± 0.16
	Sodium (Na)	7.45 ± 0.04	7.48 ± 0.07	7.68 ± 0.10	7.79 ± 0.12
	Manganese (Mn)	0.85 ± 0.02	0.87 ± 0.04	0.89 ± 0.06	0.90 ± 0.07
	Iron	6.33 ± 0.8	6.40 ± 0.09	7.68 ± 0.12	8.78 ± 0.16

Standard deviation ± error.

In the current study, the biochemical analysis of *L. vannamei* for the growth period of 40, 80, and 120 days was observed. The biochemical range was excellent including protein content and had significant results from all individual one may be attributed to increased protein been observed. It was observed that carbohydrate content exhibited an inverse relationship with protein content. It has been reported that the essential minerals were observed and plays a moderate role in the body.

Development of the pacific white shrimp (*L. vannamei*) in seven land-based ponds under zero water exchange showed the feasibility of producing of shrimp product with good growth, survival, FCR, and yield. The growth and survival of *L. vannamei* has excellent yield parameters from probiotic bacterial consortium than that the individual probiotic bacteria,

TABLE 20.7 Estimation of biochemical analysis of *Litopenaeus vannamei* culture tank for the period 120 days.

S. no.	Biochemical analysis	Quantity (mg/g) Control	*Bacillus coagulans*	*Lactobacillus acidophilus*	*B. coagulans* and *L. acidophilus*
1.	Carbohydrate	1.10 ± 0.20	1.13 ± 0.22	1.16 ± 0.18	1.18 ± 0.25
2.	Lipid	6.5 ± 0.30	7.8 ± 0.20	8.8 ± 0.24	9.11 ± 0.26
3.	Protein	116.6 ± 0.15	125.6 ± 0.26	127.8 ± 0.28	129.9 ± 0.32
4.	Ash	77.53 ± 0.39	78.56 ± 0.42	79.10 ± 1.29	82.12 ± 1.32
5.	Moisture	1.9 ± 0.08	1.13 ± 0.35	1.23 ± 0.36	1.32 ± 0.41
6.	Minerals				
	Calcium (Ca)	154.8 ± 0.28	155.9 ± 0.31	158.10 ± 0.39	161.8 ± 0.43
	Phosphorus (P)	17.64 ± 0.07	18.65 ± 0.14	18.68 ± 0.17	19.74 ± 0.20
	Potassium (K)	69.63 ± 0.19	70.73 ± 0.36	73.75 ± 0.38	76.84 ± 0.54
	Magnesium (Mg)	63.72 ± 0.08	68.8 ± 60.10	69.74 ± 0.15	73.76 ± 0.18
	Sodium (Na)	8.81 ± 0.05	8.87 ± 0.07	9.43 ± 0.10	9.89 ± 0.16
	Manganese (Mn)	0.86 ± 0.03	0.88 ± 0.05	0.92 ± 0.08	0.97 ± 0.09
	Iron	7.86 ± 0.10	8.40 ± 0.12	8.79 ± 0.14	9.86 ± 0.25

respectively. The growth and development of prawn had significant yield after incubation, respectively. Hence, the growth and development with good survival of the shrimps were observed including without pathogens, respectively.

The growth parameters of *L. vannamei* showed that ADG, final Metabolic body weight (MBW), SR, and feed conversion ratio (FCR) were a good performance of shrimp growth with zero water exchange strategy. SRs were average 90% and FCR with range 1.28–1.4, respectively. Total final biomass of shrimp varied depending on pond size and stocking density. Shrimp size at the final experiment was size range of 13.5–15.8 mm. Numerous studies showed that white shrimp, *L. vannamei*, can be raised in limited water exchange strategy with excellent health condition, good growth, low feed conversion rates, high SR, and high yield (Mishra et al., 2008). Salinity levels were slightly different with different ponds with range from 13 to 22 ppt. Location of all the earthen-ponds was far enough from the coastal area, and water resources was supplied from river and artesian well. The form of aquaculture has rain season reduced salinity due to the concentration. White shrimp, *L. vannamei*, has a good adaptation with low salinity level. The decreasing of salinity drastically made phytoplankton communities reduced so water quality changed and make shrimp stress then shrimp. Physicochemical parameters were more essential for the aquaculture industry.

The mass cultivation of *L. vannamei* with supplemented probiotic bacteria and their physicochemical parameters were analyzed. *B. coagulans* and *L. acidophilus* had maximum reduction of parameters than that of individual treated of 40, 80, and 120 days incubation by in vitro method. The physicochemical parameters like color, DO, BOD, COD, calcium, nitrate, iron and ammonia were performed with treated and control, respectively. The maximum physicochemical parameters were reduced at 120 days of incubation because of the probiotic bacteria influences on the growth and development of *L. vannamei* culture represented, respectively. The maximum growth survival of *L. vannamei* showed at 120 days after the treatment of probiotics in individually as well as combined effect of probiotics in the shrimp culture when compared with 40 days incubation, respectively. The morphometric analysis of *L. vannamei* shrimp was maximum increased when compared with control, respectively. However, the growth and development of *L. vannamei* shrimp was extraordinary production, including survival with pond ecosystem. The maximum biochemical analysis *of L. vannamei* with different probiotic bacteria were introduced and the biochemicals like carbohydrate, lipid, protein, ash, moisture, and minerals like calcium, phosphorus, potassium, magnesium, sodium, manganese, and iron content were analyzed after 40, 80, and 120 days cultured condition. Generally, combined consortium of *B. coagulans* and *L. acidophilus* had significant results recorded with conditioned environment.

16 Application of probiotics

The administration of probiotics results in the prevention of a number of physical conditions and diseases and the prophylaxis and treatment of others.

Antimicrobial compounds are synthesized by probiotics such as organic acids, hydrogen peroxide, bacteriocins, etc. Probiotics inhibit pathogens by acidification of the gut microenvironment through production of organic acids or by secretion of antimicrobial compounds (Lee, Tomita, Kleerebezem, & Bron, 2013). Pathogenic bacteria are the causative agents of bad water quality, stress, and diseases and act as a primary and secondary pathogens (Karunasagar, Otta, Karunasagar, & Joshua, 1996).

Bacteriocins: Some probiotics ply their beneficial effects by antibacterial molecules are bacteriocins that directly inhibit other bacteria or viruses, actively participating in the fight against infections, since others inhibit bacterial movement across the gut wall (translocation), enhance the mucosal barrier function by increasing the production of innate immune molecules, or modulate the inflammatory/immune response. Several studies have established that pattern recognition receptors, such as toll-like receptors signaling pathways, immune responses, and the secretion of antimicrobial peptides were defensins and chemokines by the epithelium play important roles in these mechanisms (Quigley, 2010). Here are some reports that stand as proof for the beneficial effects of probiotics. *Bacillus* S11, prior isolated from the GIT of *P. monodon* brood stock caught in the gulf of Thailand, demonstrated effective probiotic protection with *P. monodon* (Rengpipat, Phianphak, Piyatiratitivorakul, & Menasveta, 1998). After a 100-day feeding trial with probiotic supplemented and nonsupplemented (control) feeds, *P. monodon* (from PL30 onwards) exposed significant differences in growth, survival, and external appearance between the two groups. After challenging shrimps with a shrimp pathogen, *V. harveyi* by immersion for 10 days, all probiotic treated groups had 100% survival, although the control group had only 26% survival which reported competitive exclusion by probiotic *Bacillus* S11. Probiotic *B. subtilis* UTM 126 was known to produce antimicrobial activity against vibriosis in juvenile shrimp, *L. vannamei*.

Biological control: Consideration of bacterial strains preferred as probiotics should be safe to use as **biological control**. Yasuds and Taga (1980) reported that probiotic bacteria would be found to be useful not only as food but also as biological controllers of fish disease and activators of nutrient regeneration. The biological control in aquaculture did not rise, whereas the research effort has continuously increased. Generally, bacteria play two major roles as beneficial bacteria and pathogenic forms; beneficial bacteria are helpful in nutrient recycling and organic matter degradation and thus clear the environment.

Lactic acid: One of the main metabolites produced by all lactic acid bacteria is lactic acid. Acetic acid is yield only by heterofermentative lactic acid bacteria. The accumulation of lactic acid and acetic acid and the subsequent pH reduction results in a broad-spectrum inhibitory activity against Gram-positive and Gram-negative bacteria (Naidu, Bidlack, & Clemens, 1999).

17 Hydrogen peroxide (H_2O_2)

All lactic acid bacteria also produce hydrogen peroxide (H_2O_2) through electron transport via flavin enzymes. In the presence of H_2O_2, superoxide anions form destructive hydroxy radicals, thus causing peroxidation of membrane lipids and increased membrane permeability. The resulting bactericidal activity of the oxygen metabolites production is due to their strong oxidizing effect on the bacterial cell as well as destruction of nucleic acids and cell proteins (Naidu et al., 1999). H_2O_2 played antimicrobial activity against yeasts, Gram-positive and Gram-negative bacteria (Suskovich et al., 2010).

18 Influence of immune system

Probiotics also impact the immune system of the fish, shrimp, and other aquatic species. *Streptomyces* has been applied as a probiotic in the laboratory culture of *P. monodon*, which give better water quality parameters than the control tank and increased length and weight in terms of growth (Das, Lyla, & Ajmal Khan, 2006). Some probiotic products like Superbiotic, Super Ps, Zymetin, and Mutagen (Soundarapandian et al., 2010) were suggested to play a vital role in postlarvae of *P. monodon* by maintaining good water quality parameters throughout the culture period. It was reported that *B. subtilis* E20, isolated from the human health food, was used for white shrimp *L. vannamei* larvae where it displayed a significant decrease in the cumulative mortality and also increased gene expression of prophenoloxidase I, prophenoloxidase II, and lysozyme of larvae (Liu, Chiu, Shiu, Cheng, & Liu, 2010). The earlier reports displayed that for the immune power and disease resistance enhancement, the probiotics need to be given at regular intervals throughout the culture period and any halt in the probiotic supplementation leads to the untreated conditions and the animal become susceptible to the diseases. The beneficial effects of probiotics in aquaculture were illustrated in Table 20.8.

Growth promoter: Probiotics have been used in aquaculture to increase the growth of cultivated species. The use of probiotics as growth promoters of edible fishes has been recorded. Diet of Nile tilapia (*Oreochromis niloticus*) was

TABLE 20.8 Illustrate the beneficial effects of probiotics in aquaculture.

Application	Probiotic strain	Used on	References
Growth promoter	*Bacillus* sp. S11 *Lactobacillus lactis* AR21	*Penaeus monodon* *Brachionus plicatilis*	Rengpipat et al. (1998) Harzeveli, VanDuffel, Dhert, Swing, and Sorgeloos (1998)
Pathogen inhibition	*Bacillus* sp. *Lactobacillus acidophilus*	*Penaeids* *Clarias gariepinus*	Moriarty (1998) Abdullah, Hashim, and Aliyu (2011)
Nutrient digestibility	*Bacillus NL 110, Vibrio NE 17* *L. acidophilus*	*Macrobrachium rosenbergii* *C. gariepinus*	Rahiman, Yousuf, Ambat, and Hatha (2010)Dohail, Abdullah, Roshada, and Aliyu (2009).
Water quality	*Bacillus* sp. 48 *L. acidophilus*	*P. monodon* *C. gariepinus*	Wang, Li, and Lin (2008)Dohail et al. (2009)
Stress tolerance	*Bacillus subtilis* *Lactobacillus delbrueckii*	*Paralichthys olivaceus* *Dicentrarchus labrax*	Taoka, Maeda, and Jo (2006)Carnevali, de Vivo, Sulpizio, and Gioacchini (2006)
Reproduction improvement	*B. subtilis* *L. acidophilus*	*Poecilia reticulata,* *Xiphophorus hellerii*	Ghosh, Sinha, and Sahu (2007)Abasali and Mohamad (2010)

amended with a probiotic *Streptococcus* strain, highly significant the content of crude protein and crude lipid in the fish, also weight has high from 0.154 to 6.164 g in 9 weeks of culture (Lara, Olvera, Guzman, & Lopez, 2003).

Stress tolerance: Aquaculture practices demand acute productions in shorter times, causing stress in crop species. Castex, Lemaire, Wabete, and Chim (2009) reported the effect of *Pediococcus acidilactici* MA 18/5 in the antioxidative response of the shrimp *Litopenaeus stylirostris* to oxidative stress. The results gave high activities of antioxidant enzymes; superoxide dismutase (283.7 and 153.7 mol/min in control and treated groups, respectively) and catalase (3.08 and 1.34 mol/min for the control and probiotic-treated, respectively).

19 Effect of reproduction of aquatic species

Izquierdo, Palacios, and Tacon (2001) reported breeding aquaculture species have high nutritional requirements, thus reproductive capacity depends on appropriate concentrations of lipids, proteins, fatty acids, vitamins C and E, and carotenoids.

20 Conclusions

In conclusion, the specific functions of probiotics in aquaculture may not be refused. Probiotics benefits of increased disease resistance, improved nutrient digestibility and growth in the host animals, and they also improve culture water quality. Although many reports have demonstrated the efficiency of probiotics, most of these studies were conducted and evaluated under laboratory conditions. Therefore, the application of probiotics under culture conditions is necessary in order to accurately evaluate their use.

The importance of various probiotics in improving the growth and survivability of *Penaeus vannamei* postlarvae is studied. Better performance was observed in groups fed with probiotic incorporated diets like combination of *B. subtilis* and *L. acidophilus* than individual when compared to control. Probiotic treatment has also shown to elicit the cellular and humoral immunity, thus explaining the mechanism of action of these probionts. Hence, we propose that the probiotics, particularly combination of *B. subtilis* and *L. acidophilus*, have the potential to be used in shrimp culture owing to their beneficial effects. Furthermore, study is warranted to clearly understand the target pathways and the mechanism of action of probiotics in shrimps. It provides a basis for the further development of remediation techniques to reduce the environmental impact of shrimp aquaculture. For sustainable and healthy development of shrimp farming, a new shrimp-breeding model that is environmentally friendly and self-healing is urgently needed.

Acknowledgments

The authors want to thank Secretary and Principal of A.V.V.M. Sri Pushpam College (Autonomous), Poondi-613 503 Thanjavur and anonymous reviewers for their valuable suggestions to improve this review.

References

Abasali, H., & Mohamad, S. (2010). Effect of dietary supplementation with probiotic on reproductive performance of female livebearing ornamental fish. *Research Journal of Animal Sciences*, *4*(4), 103–107.

Abdullah, A. M., Hashim, R., & Aliyu, P. M. (2011). Evaluating the use of *Lactobacillus acidophilus* as a biocontrol agent against common pathogenic bacteria and the effects on the haematology parameters and histopathology in African catfish *Clarias gariepinus* juveniles. *Aquaculture Research*, *42*(2), 196–209.

Mondal, T. J. S., Abraham, S., & Babu, C. S. (2008). Effect of commercial aquaculture probiotic and fish gut antagonistic bacterial flora on the growth and disease resistance of ornamental fishes *Carassius auratus* and *Xiphophorus hellerii*. *European Union Journal of Fisheries & Aquatic Sciences*, *25*(1), 27–30.

Allan, G. L., & Maguire, G. B. (1992). Effect of stocking density on production of *Penaeus monodon* model farming systems. *Aquaculture*, *107*, 49–66.

Apha (1992). In *Standard methods for the examination of water and wastewaters* (18th ed.). Washington, DC: American Public Health Association. p. 1268.

Balcazar, J. L., de Blas, I., Ruiz-Zarzuela, I., Cunningham, D., Vendrell, D., & Muzquiz, J. L. (2006). The role of probiotics in aquaculture. *Veterinary Microbiology*, *114*, 173–186.

Ballester, E. L. C., Wasielesky, W. J., Cavalli, R. O., & Abreu, P. C. (2007). Nursery of the pink shrimp *Farfantepenaeus paulensis* in cages with artificial substrates: Biofilm composition and shrimp performance. *Aquaculture*, *265*, 355–362.

Bandyopadhyay, P., & Mohapatra, P. K. D. (2009). Effect of a probiotic bacterium *Bacillus circulans* PB7 in the formulated diets: On growth, nutritional quality and immunity of *Catla catla* (Ham). *Fish Physiology and Biochemistry*, *35*, 467–478.

Brownell, C. L. (1980). Water quality requirements for first feeding in marine fish larvae: Ammonia, nitrite and nitrate. *Journal of Experimental Marine Biology and Ecology*, *44*(2), 269–283.

Carnevali, O., de Vivo, L., Sulpizio, R., & Gioacchini, G. (2006). Growth improvement by probiotic in European sea bass juveniles (*Dicentrarchus labrax* L.), with particular attention to IGF-1, myostatin and cortisol gene expression. *Aquaculture*, *258*(1–4), 430–438.

Castex, M., Lemaire, P., Wabete, N., & Chim, L. (2009). Effect of dietary probiotic *Pediococcus acidilactici* on antioxidant defences and oxidative stress status of shrimp *Litopenaeus stylirostris*. *Aquaculture*, *294*(3–4), 306–313.

Chantharasophon, K., Warong, T., Mapatsa, P., & Leelavatcharamas, V. (2011). High potential probiotic *Bacillus* species from gastrointestinal tract of Nile tilapia (*Oreochromis niloticus*). *Biotechnology*, *4*(6), 498–505.

Chu, W., Lu, F., Zhu, W., & Kang, C. (2011). Isolation and characterization of new potential probiotic bacteria based on quorum-sensing system. *Journal of Applied Microbiology*, *110*, 202–208.

Coeuret, V., Guwguen, M., & Vernoux, J. P. (2004). Numbers and strain of lactobacilli in some probiotic products. *International Journal of Food Microbiology*, *97*, 147–156.

Dalmin, G., Kathiresan, K., & Purushothaman, A. (2001). Effect of probiotics on bacterial population and health status of shrimp in culture pond ecosystem. *Indian Journal of Experimental Biology*, *39*, 939–942.

Das, S., Lyla, P. S., & Ajmal Khan, S. (2006). Application of *Streptomyces* as a probiotic in the laboratory culture of *Penaeus monodon* (Fabricius). *Israeli Journal of Aquaculture*, *58*(3), 198–204.

Dohail, A., Abdullah, M., Roshada, H., & Aliyu, M. (2009). Effects of the probiotic, *Lactobacillus acidophilus*, on the growth performance, haematology parameters and immunoglobulin concentration in African Catfish (*Clarias gariepinus*, Burchell 1822) fingerling. *Aquaculture Research*, *40*(14), 1642–1652.

Ebeling, J. M., Timmons, M. B., & Bisogni, J. J. (2006). Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic control of ammonia nitrogen in aquaculture production systems. *Aquaculture*, *257*, 346–358.

FAO/WHO. (2001). *Report of a Joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria.* (pp. 2–8). Canada: FAO/WHO.

Fast, A. W., & Lannan, J. E. (1992). Pond dynamic processes. In A. W. Fast, & J. Lester (Eds.), *Marine shrimp culture: Principles and practices* (pp. 431–455). Amsterdam: Elsevier Science Publication.

Fernandez, R., Sridhar, M., & Sridhar, N. (2011). Effect of lactic acid bacteria administered orally on growth performance of *Penaeus indicus* (H. Milne Edwards) juveniles. *Research Journal of Microbiology*, *6*(5), 466–479.

Fuller, R. (1989). A review probiotic in man and animals. *Journal of Applied Environmental Microbiology*, *63*(1), 1034–1039.

Gatesoupe, F. J. (2005). Probiotics and prebiotics for fish culture, at the parting of the ways. *Aqua Feeds Formulation Beyond*, *2*(3), 3–5.

Ghosh, S., Sinha, A., & Sahu, C. (2007). Effect of probiotic on reproductive performance in female livebearing ornamental fish. *Aquaculture Research*, *38*(5), 518–526.

Gilles, L. M. (2001). Environmental factors affect immune response and resistance in *Crustaceans*. *The Advocate*, *18*, 1–18.

Gunalan, B., Soundarapandian, P., & Dinakaran, G. K. (2010). Effect of probiotics on growth and survival of *Penaeus mondon* (Fabricius). *Asian Journal of Agricultural Sciences*, *2*(1), 5–8.

Harzeveli, A. R. S., VanDuffel, H., Dhert, P., Swing, J., & Sorgeloos, P. (1998). Use of a potential probiotic *Lactococcus lactis* AR21 strain for the enhancement of growth in the rotifer *Brachionus plicatilis* (Muller). *Aquaculture Research*, *29*(6), 411–417.

Hossain, M. I., Kamal, M. M., Mannan, M. A., Bhuyian, M. A. B., & Hossain, M. I. (2013). Effects of probiotics in growth and survival of shrimp (*Penaeus monodon*) in coastal pond at Khulna. *Bangladesh Journal of Scientific Research*, *5*(2), 363–370.

Izquierdo, M., Palacios, H., & Tacon, A. (2001). Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture*, *197*(1–4), 25–42.

Juntunen, M., Kirjavainen, P. V., Ouwehand, A. C., Salminen, S. J., & Isolauri, E. (2001). Adherence of probiotic bacteria to human intestinal mucus in healthy infants and during rotavirus infection. *Clinical and Diagnostic Laboratory Immunology*, *8*, 293–296.

Karthikeyan, J. (1994). Aquaculture (Shrimp farming) its influence on environment. In *Technical paper submitted to the seminar 'Our Environment-its challenges to development projects'*American Civil Engineers, Calcutta, India, 9–10 September.

Karunasagar, I., Otta, S. K., Karunasagar, I., & Joshua, K. (1996). Applications of *Vibrio* vaccine in shrimp culture. *Fishing Chimes*(16), 49.

Kesarcodi-Watson, A., Kaspar, H., Lategan, M. J., & Gibson, L. (2008). Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. *Aquaculture*, *274*, 1–14.

Kuhn, D. D., Boardman, G. D., Marsh, L., Lawrence, A. L., & Flick, G. J. (2009a). Technology and research advances for the production of marine shrimp in recirculating aquaculture systems. *Journal of Shellfish Research*, *28*, 709.

Lakshmi, B. V., & Sai Gopal, D. V. R. (2013). Probiotics as antiviral agents in shrimp aquaculture. *Journal of Pathogens*, 1–13. http://dx.doi.org/10.1155/2013/424123.

Lara, F., Olvera, N., Guzman, M., & Lopez, M. (2003). Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, *216*(1–4), 193–201.

Lara-Flores, M., & Aguirre-Guzman, G. (2009). The use of probiotic in fish and shrimp aquaculture (a review). In N. Perez-Guerra, & L. Pastrana-Castro (Eds.), *Probiotics: Production, evaluation and uses in animal feed* (pp. 75–89). Kerala: Research Signpost.

Lee, I. -C., Tomita, S., Kleerebezem, M., & Bron, P. (2013). The quest for probiotic effector molecules unraveling strain specificity at the molecular level. *Pharmacological Research*, *69*, 61–74.

Lilly, D. M., & Stillwell, R. H. (1965). Probiotics: Growth-promoting factors produced by microorganisms. *Science*, *147*(3659), 747–748.

Liu, K. F., Chiu, C. H., Shiu, Y. L., Cheng, W., & Liu, C. H. (2010). Effects of the probiotic, *Bacillus subtilis* E20, on the survival, development, stress tolerance, and immune status of white shrimp, *Litopenaeus vannamei* larvae. *Fish and Shellfish Immunology*, *28*(5–6), 837–844.

Mishra, J. K., Samocha, T. M., Patnaik, S., Speed, M., Gandy, R. L., & Ali, A. (2008). Performance of an intensive nursery system for the Pacific white shrimp, *Litopenaeus vannamei*, under limited discharge condition. *Aquacultural Engineering*, *38*, 2–15.

Moriarty, D. J. W. (1998). Control of luminous *Vibrio* species in penaeid aquaculture ponds. *Aquaculture*, *164*, 351–358.

Mukherjee, A., Dutta, D., Banerjee, S., Ringo, E., Breines, E. M., Hareide, E., et al. (2016). Potential probiotics from Indian major carp, *Cirrhinus mrigala.* Characterization, pathogen inhibitory activity, partial characterization of bacteriocin and production of exoenzymes. *Research in Veterinary Science*, *108*, 76–84.

Mustafa, S., & Ridzwan, A. R. (2000). *Sustainable marine aquaculture recent developments with special reference to Southeast Asia*. Malaysia: Kota Kinabalu.

Muthu, M. S. (1980). Site selection and type of farms for coastal aquaculture of prawns. In *Proceedings of the symposium on shrimp farming* (pp. 97–106). Marine Products Export Development Authority, Bombay, 16–18 August.

Naidu, A. S., Bidlack, W. R., & Clemens, R. A. (1999). Probiotic Spectra of Lactic Acid Bacteria (LAB). *Critical Reviews in Food Science and Nutrition*, *38*(1), 13–126.

Nayak, A. K., Reddy, A. K., & Pawar, R. A. (2003). Use of probiotics in aquaculture. *Fishing Chimes*, *22*(10/11), 40–43.

Ngan, P. T. T., & Phu, T. Q. (2011). Effects of *Bacillus* bacteria (B8, B37, B38) on water quality of black tiger shrimp (*Penaeus monodon*) cultured tanks. In *Proceedings of the 4th aquaculture and fisheries conference* (pp. 28–41). .

Ouwehand, A. C., Tolkko, S., Kulmala, J., Salminen, S., & Salminen, E. (2000). Adhesion of inactivated probiotic strains to intestinal mucus. *Letters in Applied Microbiology*, *31*, 82–86.

Pandiyan, P., Balaraman, D., Thirunavukkarasu, R., George, E. G. J., Subaramaniyan, K., Manikkam, S., et al. (2013). Probiotics in aquaculture. *Drug Invention Today*, *5*, 55–59.

Parker, P. B. (1974). Probiotics, the other half of the antibiotic story. *Animal Nutrition and Health*, *29*, 4–8.

Prasad, L., Baghel, D. S., & Kumar, V. (2003). Role and prospects of probiotics use in aquaculture. *Aquaculture*, *4*(2), 247–251.

Quigley, E. M. M. (2010). Prebiotics and probiotics; modifying and mining the microbiota. *Pharmacological Research*, *61*(3), 213–218.

Rahiman, M., Yousuf, J., Ambat, T., & Hatha, M. (2010). Probiotic effect of *Bacillus* NL110 and *Vibrio* NE17 on the survival, growth performance and immune response of *Macrobrachium rosenbergii* (de Man). *Aquaculture Research*, *41*(9), e120–e134.

Ramanathan, N., Padmavathy, P., Francis, T., Athithian, S., & Selvaranjitham, N. (2005). *Manual on polyculture of tiger shrimp and carps in freshwater*. Thoothukudi: Tamil Nadu Veterinary and Animal Sciences University, Fisheries College and Research Institute. pp. 1–161.

Rao, V. A. (2002). Bioremediation technology to maintain healthy ecology in aquaculture ponds. *Fishing Chimes*, *22*(6), 39–42.

Ravi, V. S., Ajmalkhan, S., & Rajagopal, S. (1998). Influence of probiotics on growth of Indian white prawn, *Penaeus indicus*. *Journal of Scientific and Industrial Research*, *57*, 752–756.

Rengpipat, S., Phianphak, W., Piyatiratitivorakul, S., & Menasveta, P. (1998). Effects of a probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. *Aquaculture*, *167*(3–4), 301–313.

Rengpipat, S. (2005). Biocontrol of bacteria pathogens in aquaculture with emphasis on change therapy. In P. Walker, R. Lester, & M. G. Bondad-Reantaso (Eds.), *Diseases in Asian aquaculture* (pp. 543–552). Manila: Fish Health Section, Asian Fisheries Society.

Ringo, E., Strom, E., & Tabachek, J. A. (1995). Intestinal microflora of salmonids (a review). *Aquaculture Research*, *26*, 773–789.

Ringo, E., Salinas, I., Olsen, R. E., Nyhaug, A., Myklebust, R., & Mayhew, T. M. (2007). Histological changes in intestine of Atlantic salmon (*Salmo salar* L.) following in vitro exposure to pathogenic and probiotic bacterial strains. *Cell and Tissue Culture Research*, *328*, 109–116.

Sakata, T. (1990). Microflora in the digestive tract of fish and shellfish. In R. Lesel (Ed.), *Microbiology in poecilotherms* (pp. 171–176). Amsterdam: Elsevier.

Sambhu, C., & Jayaprakas (2001). Livol (IHF-1000), a new herbal growth promoter in white prawn, *Penaeus indicus* (Crustacea). *Indian Journal of Marine Sciences*, *30*, 38–43.

Samocha, T. M., Lawrence, A. L., & Pooser, D. (1998). Growth and survival of juvenile *Penaeus vannamei* in low salinity water in a semi-closed recirculating system. *Israeli Journal of Aquaculture – Bamidgeh*, *50*(2), 55–59.

Soundarapandian, P., & Gunalan, B. (2008). Recent technology for the survival and production of giant tiger shrimp *Penaeus monodon* along south east coast of India. *International Journal of Zoology and Research*, *4*(1), 21–27.

Soundarapandian, P., Ramanan, V., & Dinakaran, G. K. (2010). Effect of probiotics on the growth and survival of *Penaeus monodon* (Fabricius). *Current Research Journal of Social Sciences*, *2*(2), 1–57.

Sun, Y. Z., Yang, H. L., Ma, R. L., Song, K., & Li, J. S. (2012). Effect of *Lactococcus lactis* and *Enterococcus faecium* on growth performance, digestive enzymes and immune response of grouper *Epinephelus coioides*. *Aquaculture Nutrition*, *18*, 281–289.

Suskovich, J., Kos, B., Beganovich, J., Pavunc, A., Habjanic, K., & Matosich, S. (2010). Antimicrobial activity – The most important property of probiotic and starter lactic acid bacteria. *Food Technology and Biotechnology*, *48*(3), 296–307.

Tannock, G. W. (1997). Probiotic properties of Lactic acid bacteria: Plenty of scope for fundamental R&D. *Trends in Biotechnology*, *15*(7), 270–274.

Taoka, Y., Maeda, H., Jo, J., Jeon, M., Bai, S. C., Lee, W., et al. (2006). Growth, stress tolerance and non-specific immune response of Japanese flounder *Paralichthys olivaceus* to probiotics in a closed recirculating system. *Fisheries Science*, *72*(2), 310–321.

Tsai, S. J., & Chen, J. C. (2002). Acute toxicity of nitrate on *Penaeus monodon* juveniles at different salinity levels. *Aquaculture*, *213*(1–4), 163–170.

Vaseeharan, B., & Ramasam, P. (2003). Control of pathogenic *Vibrio* spp. by *Bacillus subtilis* BT23, a possible probiotic treatment for black tiger shrimp *Penaeus monodon*. *Letters in Applied Microbiology*, *36*(2), 83–87.

Wang, W. N., Wang, A. L., Zhang, Y. J., Li, Z. H., Wang, J. X., & Sun, R. Y. (2004). Effects of nitrite on lethal and immune response of *Macrobrachium nipponense*. *Aquaculture*, *232*(2004), 679–686.

Wang, Y. B., Li, J. R., & Lin, J. (2008). Probiotics in aquaculture: Challenges and outlook. *Aquaculture*, *281*(1–4), 1–4.

Wen-Young, T. (1998). *Shrimp mariculture: A practical manual* (2nd ed.). Brisbane, Australia: W.S. Aquaculture, Cannan International Pty. Ltd. p. 282.

Yasuds, K., & Taga, N. (1980). A mass culture method for *Artemia salina* using bacteria as food. *Medical Education Resources*, *18*, 53.

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Chapter 21

Prospective Approaches of *Pseudonocardia alaniniphila* Hydrobionts for *Litopenaeus vannamei*

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1 Introduction

Mangroves are salt tolerant vegetation that grows in intertidal regions of rivers and estuaries. Sediments deposition and availability of sheltered areas are two essential prerequisites for establishment of mangroves. Mangroves are also referred to as tidal forests and belong to the category of tropical wetland rain forest ecosystem. Mangrove occurs almost exclusively in the tropical regions of the world, covering an area of around 200,000 km^2 in 30 countries. Mangroves are unique forests representing intermediate vegetation between land and sea that grow in oxygen deficient water soils. To survive in such harsh conditions, mangroves have evolved a number of physiological and structural adaptations like vivipary, pneumatophores, properties salt secretion, and ultrafiltration. All mangrove species have the mechanism to provide air to their root systems from the atmosphere. Hence, they can tolerate anaerobic conditions to some extent. Ecologically, mangroves are colonizers, creating hand ferns out of sea. Mangroves perform a large number of vital ecological functions like nutrient recycling, maintenance of hydrological region, coastal protection, fish, and fauna production, which are vital for the survival of both man and animals. However, human depending on mangrove resources has claimed heavily on its areas and function. As mangroves occur mostly in the tropical region where majority of the world population reside, they have been gradually cleared through the years to meet the needs of the burgeoning population.

Microbial diversity and composition are important for the maintenance of mangrove ecosystem. Analysis of microbial biodiversity from this ecosystem will help in isolating and identifying new and potent microorganisms having high specificity for various applications.

1.1 Actinobacteria

Actinobacteria are a group of branching unicellular organisms that are produced either by fission or by means of special spores or conidia. They are closely related to bacteria; frequently, they are considered as higher filamentous bacteria. Marine Actinobacteria are probiotics in aquaculture. Das, Ward, and Burke (2008) began screening marine Actinobacteria for use as new biocontrol agents for aquatic animals.

1.2 Aquaculture

India is the second-largest producer of aquatic animals globally. The majority of the aquaculture growth in India has taken place in the 21st century, driven by an almost 150% growth in the production of carps to 4.2 million metric tons (FAO). Additionally, India has developed a vibrant shrimp farming sector that is ranked 4th globally after China, Indonesia, and

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00021-6
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Vietnam. In India, aquaculture sector has turned into a fast-growing commercial sector. Aquaculture, probably the fastest growing food-producing sector, presents the greatest potential to meet demands for aquatic food supply. However, in order to accomplish these goals, the sector will face significant challenges to increase aquaculture profitability.

Cultured species depend on our knowledge of nutrition, biochemistry, physiology and genetics, among others. Feeding represents 40%–60% of total production cost in shrimp farms; therefore, new varieties of feed formulations must be directed to be well-balanced and inexpensive diets (Bautista, Eusebio, & Welsh, 2003).

1.3 Shrimp diseases

Infectious diseases in shrimp industry are caused by viral, bacterial, and eukaryote pathogens as well as some abiotic factors. Infectious diseases have become a stumbling block for expansion of shrimp aquaculture due to losses recorded (Stentiford et al., 2012). Most of these losses have been attributed to viruses, which accounts for 60%, and an estimated 20% has been attributed to bacteria while eukaryotes such as fungi and parasites losses are comparably low (Flegel, 2012). Shrimp diseases caused by opportunistic bacteria such as *Vibrio* sp. and viruses are the major problems that can lead to huge losses in farmed shrimp industry (Moriarty, 1999b).

Shrimp viral diseases that account for almost 60% of all shrimp losses in shrimp aquaculture are caused mainly by some viruses, namely, infectious hypodermal and hematopoietic necrosis virus, Taura syndrome virus, white spot syndrome virus, yellow head virus, and infectious myonecrosis virus (Seibert & Pinto, 2012). Most viral diseases proliferate and make substantive impact when wild-caught brood stock are used in hatchery production to supply millions of larvae to stock the ponds and importation of live shrimp for aquaculture (Karunasagar & Ababouch, 2012). Unlike viruses, bacterial diseases of shrimp are mostly caused by various species of *Vibrio* (Moriarty, 1999a). In most diseased shrimp studies, *Vibrio* species are the frequent bacterial flora that causes vibriosis. The pathogens have led to the collapse of many farms due to its severe effect on hatcheries and high rate of morbidity and mortality. In the world of shrimp aquaculture, vibriosis has been a major problem and capable of crippling shrimp culture ponds with consequent huge economic losses (Yanbo & Qing, 2015) The several diseases caused by *Vibrio* species include loose shell syndrome (LSS), white gut disease (WGD), tail necrosis, shell disease and red disease of which LSS, WGD, and red disease caused mass mortalities in shrimp culture ponds (Jayasree, Janakiram, & Madhavi, 2006).

1.4 Control of shrimp pathogen

Control of shrimp bacterial infections has relied solely on the use of antibiotics and antimicrobial disinfectants or chemical additives. Biofilm formation allows *Vibrio* species to thrive on surfaces even in the presence of antibiotics which also pose a challenge in the management of shrimp diseases by protecting the bacteria against antibiotics (Karunasagar & Ababouch, 2012). The use of chemical agents has also been challenged due to the resultant adverse environmental effects (Wang & Xu, 2004). To effectively manage shrimp diseases, alternative control and management measures are required (Moriarty, 1999a).

1.5 Probiotics: definition and principles

Probiotics that compete with bacterial pathogens for nutrients or inhibit the growth of pathogens can be a valid alternative to the prophylactic application of antibiotics and biocides. Probiotic are defined as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" (Fuller, 1989). Probiotics, as one alternative to antibiotics, have been commonly administered in aquaculture because they confer several benefits including immunological, nutritional, and environmental benefits (Lazado, Caipang, & Estante, 2015).

Bacillus probiotic strains have been introduced to improve *Litopenaeus vannamei* growth performance, digestive enzyme activity and immune response, getting good results in all the parameters measured. However, the 100% replacement of fecal microbiota (FM) by soyabean meal (SBM) and high levels of complex carbohydrates (CHOs) has never been done successfully with and without *Bacillus* strains. The maximum amount of SBM included in *L. vannamei* experimental diets without health problems reach 20% but always in combination with 20% or more of FM inclusions (Shen, Fu, Li, & Zhu, 2010).

2 Materials and methods

2.1 Study site

Muthupet, meaning land of pearls, is the biggest mangrove forest in Tamil Nadu. It is located in 10.3957°N, 79.4950°E along the Palk Strait covering an area of 11,885.91 ha in Manakkattai, Lagoon, and Xaviermunai of Thiruvarur and

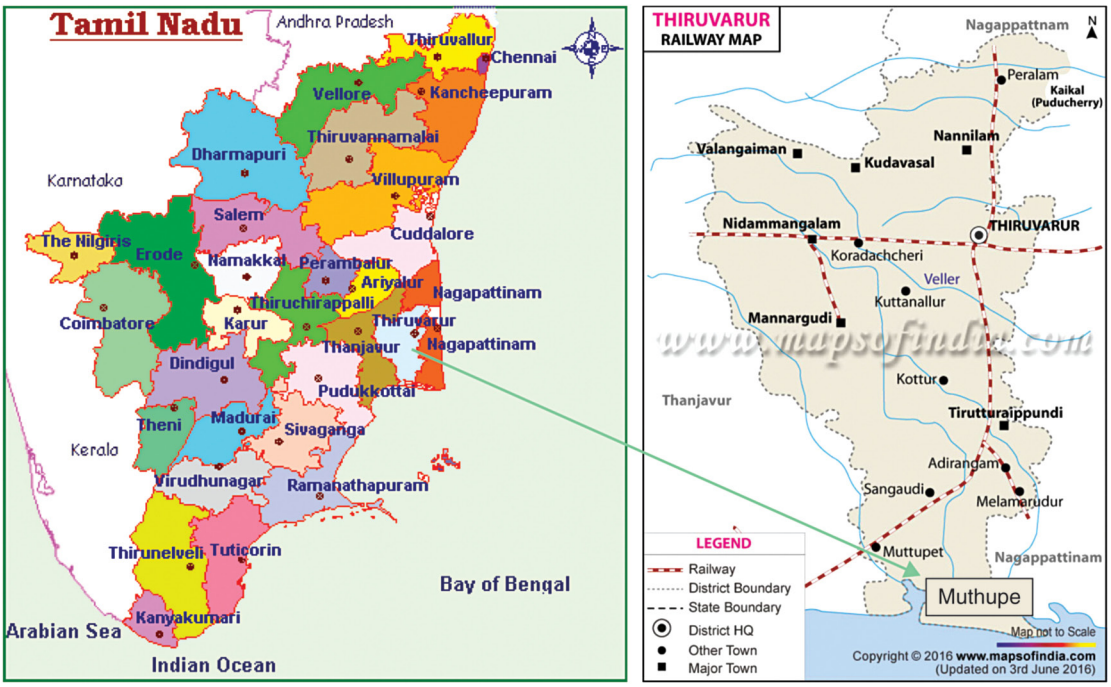


FIGURE 21.1 Study site.

Thanjavur districts. The soil samples were collected from Muthupet area, namely, Koraiyar, Manakkattai, Lagoon, and Xaviermunai in Thiruvarur district (Fig. 21.1). Four rivers of the Cauvery delta, namely, Paminiyar, Koraiyar, Kilaithankiyar, and Marakkakoraiyar, flow through the Muthupet mangroves.

2.2 Sample collection

Mangrove soil samples were collected from different sites of mangrove environments in Muthupet, Thiruvarur district, Tamil Nadu. The soil samples were collected from 10 to 15 cm depth during April 2014. The soil samples were collected from sterilized bottle and to bring laboratory for further investigation (Fig. 21.2).

2.3 Analysis of physicochemical parameters in soil

The collected samples were analyzed for different physicochemical parameters soil texture was estimated in sieving process, and pH and electrical conductivity were measured by using a digital pH and conductivity meter (Systronics, 306); cation exchange capacity, organic carbon, organic matter, nitrogen, phosphorus, potassium, calcium, magnesium, sodium, zinc, copper, iron, and manganese were also analyzed.

2.4 Isolation and identification of Actinobacteria

Isolation of Actinobacteria was done by serial dilution and spread plate method (Oskay & Husniye, 2015). One gram soil sample were taken and dissolved in 100 mL of distilled water (10^{-1}), then completely shaken and 1 mL of diluted samples transferred in 10 mL of distilled water, they denotes the test tube 10^{-2} and diluted up to 10^{-7} factors. To prepare Starch Casein Agar (SCA) (soluble starch, 10.0 g; casein hydrolysate, 0.3 g; KNO_3, 2.0 g; NaCl, 2.0 g; K_2HPO_4, 2.0 g; $MgSO_4{\cdot}7H_2O$, 0.05 g; $CaCO_3$, 0.02 g; $FeSO_4{\cdot}7H_2O$, 0.01 g; agar, 18.0 g; distilled water, 1000 mL; pH 7; cycloheximide, 100 µg/mL) medium and 0.1 mL of suitable dilution (10^{-2}, 10^{-3}, and 10^{-4}) spread over the SCA medium plate individually, all the plates were incubated at 30°C for 1–2 weeks, after they calculated in colony forming unit per 1 g of soil samples.

After the incubation period, the colonies were identified based on the morphological and biochemical characterization using standard procedure then referred by Bergey's manual of systematic bacteriology of 9th edition.



FIGURE 21.2 Sample collection.

2.5 Determination of antimicrobial activity

2.5.1 Test microbes

The test bacterial strains are *Escherichia coli* (MTCC1687), *Pseudomonas aeruginosa* (MTCC424), *Staphylococcus aureus* (MTCC737), and *Salmonella typhi* (MTCC98); fungal strains as *Aspergillus flavus* (MTCC9606), *Aspergillus fumigatus* (MTCC2584), *Aspergillus terreus* (MTCC1782), and *Penicillium* sp. (MTCC5108), and shrimp pathogen, namely, *Vibrio alginolyticus* (MTCC4442), *Vibrio parahaemolyticus* (MTCC451), *Vibrio harveyi* (MTCC3438), and *Vibrio vulnificus* (MTCC1145) were procured from Microbial Type Culture Collection and Gene Bank (MTCC) at CSIR—Institute of Microbial Technology, Chandigarh, for further studied.

2.5.2 Agar well diffusion method

Agar well diffusion method was followed for antimicrobial activity. Nutrient agar is used for determining antibacterial activity and potato dextrose agar for antifungal activity. The human, bacteria and fungi, and shrimp pathogen strains freshly prepared in broth form were used for antimicrobial determination. The respective strains were swabbed (sterile cotton swabs) in susceptible media. Then, agar wells (5-mm diameter) were made in each of these plates using sterile cork borer. Different strains (AVVM1 to AVVM11) of Actinobacteria were added using sterilized dropping pipettes into the wells, and plates were left for 1 hour allowed for preincubation. The plates were incubated at 37°C for 24 hours, and the inhibition zone was measured and calculated. The results were compared to standard antibiotics (A^{25}—ampicillin, C^{30}—chloramphenicol, CIP^{5}—ciprofloxacin, E^{5}—erythromycin, GEN^{10}—gentamycin, P^{10}—penicillin, PB^{300}—polymyxin, S^{25}—streptomycin, and RIF^{5}—rifampicin).

2.6 Molecular characterization of potential Actinobacteria

2.6.1 DNA Isolation and 16S rRNA amplification

For molecular identification of Actinobacteria, the genomic DNA was isolated by using DNA extraction kit (Marmur, 1961). Polymerase chain reaction products were generated with the primer pair 8F (5′-AGAGATTTGATCCTGGCTCAG-3′) and 338R (5′-TGCTGCCTCCCGTAGGAGT-3′), which amplified the V1 and V2 regions of the 16S rRNA gene. The amplified polymerase chain reaction (PCR) product was checked by using 1.2% agarose gel electrophoresis using TAE buffer. The PCR bands were analyzed under UV light and documented using a Bio-Rad Gel Doc XR+ system (Hercules, CA, United States). The PCR products of 16S rRNA gene was purified by quick PCR purification kit (Invitrogen), and sequencing was done commercially at SciGenome Pvt. Ltd., Kochi, India.

After purification, the PCR amplicons were directly sequenced by DNA sequencing Services, Malaysia (Deshmukh & Sridhar, 2002). The sequence was compared for similarity level with the reference strains of Actinobacteria from genomic database banks, using the NCBI Blast available at the http://www.ncbi.nlm.nih.gov/ blast web site. Phylogenetic distance trees were inferred by Neighbor-Joining analyses using MEGA 5.10 (Tamura et al., 2011). Confidence in topologies was assessed using bootstrapping (1000 replicates).

The secondary structure of 16SrRNA gene of potential SVVA7, SVVA14, and SVVA22 strain was predicted by using the bioinformatics tool available in online http://rna.urmc.rochester.edu/RNA Structure Web/servers/Predict.

2.7 Experimental design and feeding management

SPF (Specific Pathogenic Free) *L. vannamei* shrimps (DOC 50, ABW 6–7 g) were collected from Lakshmi aqua farm, Eripurakarai, Adirampattinam, Tamil Nadu, India (Boonyaratpalin, 1993).

The shrimp were fed with CP commercial feed (BLANCA shrimp feed, CP company) and had a crude protein 35%, fat 5%, and fiber 4%. Uniform size of *L. vannamei* shrimps were collected from the stock catchery and transferred in individual experimental FRP (fiberglass-reinforced plastic) circular tanks. Five tanks (142.5-cm diameter, 90-cm height, and1500-L capacity) were chosen in the experimental design, and the treatments are as follows:

	Diet composition (g/kg)				
Treatments	**CP Feed (g)**	**SVVA22 (g)**	**VIT-C (g)**	**β-Glucan (g)**	**FOS (g)**
Tank-1		–	–	–	–
Tank-2		5	–	–	–
Tank-3	1000	5	5	–	–
Tank-4		5	–	5	–
Tank-5		5	–	–	5

Explanation of diet

CP Feed	:	BLANCA shrimp feed, CP India Pvt. Ltd., Red Hills, TN, India
SVVA22	:	*Pseudonocardia alaniniphila* AVVM03 (MN558974)
VIT-C	:	ENVITA C (Biovedic Nutraceuticals, Bengaluru)
β-Glucan	:	ALETA (Kemin Industries South Asia Pvt. Ltd., Chennai)
FOS	:	NEOSUGAR (Meiji Seika Kaisha Ltd., Japan)

Each treatment and control groups was maintained with replicates. The shrimps were fed a day at 7.00, 11.00 and 14.00 and 17.00 hours at 10% of the body weight. The culture tanks were cleaned manually once in 2 days to remove uneaten feed and waste matters. Then 80% of water was exchanged at 30-day intervals.

2.8 Analysis of physicochemical parameters in water samples

The physicochemical parameters like color, temperature, pH, turbidity, salinity, alkalinity, total hardness, dissolved oxygen, biological oxygen demand, chemical oxygen demand, calcium, nitrate, nitrite, iron, and ammonia were analyzed by standard methods (APHA; AWWA; WPCF, 1985).

2.9 Growth and development of *Litopenaeus vannamei*

Shrimp length measurement, weight gain, average daily growth, specific growth rate, and feed conversion ratio (FCR) were calculated and statistically analyzed (ANOVA), and the percentage of survival rate (SR), total feed consumption, and total yield were estimated (Jayaram, 1981). The following growth parameters were used for studying the growth performance of *L. vannamei* as recorded in DOC (Day of Culture) at 50, 75, and 100days.

Growth parameters are as follows:

1. Length measurement $= \dfrac{\text{final length}(\text{cm}) - \text{initial length}(\text{cm})}{\text{initial length}(\text{cm})}$

2. Weight gain $= \dfrac{\text{final weight}(\text{g}) - \text{initial weight}(\text{g})}{\text{initial length}(\text{cm})}$

3. Average daily gain $(\text{g}) = \dfrac{\text{final weight}(\text{g})}{\text{duration of culture}(\text{days})}$

4. Specific growth rate $= \dfrac{\text{Ln}\left(\text{final weight}(\text{g})\right) - \ln\left(\text{initial weight}(\text{g})\right)}{\text{duration of culture}(\text{days})}$

5. Survival rate $\% = \dfrac{\text{no of shrimp live}}{\text{no of shrimp stocked}} \times 100$

6. Feed conversion ratio $= \dfrac{\text{total feed fed}(\text{dry wt.})(\text{kg})}{\text{total yield}(\text{kg})}$

2.10 Biochemical analysis

The crude protein, crude lipid, and CHO were estimated as per standard method (AOAC, 1990). One gram of powdered shrimp tissue was taken in a porcelain crucible was kept in a muffle furnace at 60°C for 4 hours. The residue ash content was weighed, and the percentage was calculated. Moisture content was estimated by hot air oven method and minerals were analyzed.

2.11 Immunological studies

Coagulase activity, total hemocyte count (THC) (Neubauer's Hemocytometer), intracellular anion, and prophenoloxidase enzyme assay were estimated by spectrophotometer method (Peters & Long, 1973).

3 Results and discussion

The present investigation focused on the screening of potential probiotic Actinobacteria from mangrove ecosystem and used for the probiotic with prebiotics supplemented with *L. vannamei*.

TABLE 21.1 Analysis of physiochemical properties of soil samples from different place of Muthupet mangroves environments (April 2014).

Name of the parameters	Study area			
	Koraiyar	Manakkattai	Lagoon	Xaviermunai
Soil texture				
Clay (%)	28	25	26	21
Slit (%)	9	7	10	9
Find sand (%)	21	28	23	26
Core sand (%)	42	40	41	44
pH	7.90	8.17	8.58	8.51
Electrical conductivity (dS/m)	2.21	1.96	2.36	2.48
Cation exchange capacity (C mol $Proton^+$ kg)	21.7	20.5	27.2	23.4
Organic carbon (%)	0.19	0.31	0.41	0.28
Organic matter (%)	0.39	0.62	0.72	0.56
Nitrogen (mg/kg)	11.05	9.33	9.20	0.79
Phosphorus (mg/kg)	7.22	6.24	6.52	6.03
Potassium (mg/kg)	10.2	1.56	2.99	1.12
Calcium (mg/kg)	12.3	13.2	12.0	11.0
Magnesium (mg/kg)	11.0	9.37	10.3	9.02
Sodium (mg/kg)	5.32	2.20	4.36	5.21
Zinc (ppm)	0.97	1.45	1.27	1.75
Copper (ppm)	0.65	0.71	0.53	0.68
Iron (ppm)	8.76	7.77	9.22	7.14
Manganese (ppm)	2.26	3.23	3.55	2.78

3.1 Physicochemical properties of soil sample

The soil samples were collected from Muthupet, east coast of mangroves, Tamil Nadu, India during April 2014. The physiochemical parameters and soil samples were analyzed and tabulated (Table 21.1).

3.2 Soil texture

In soil sediment texture analyzed, coarse sand was dominant in all the stations followed by clay, fine sand, and silt. Coarse sand ranged between 40% and 44%, clay ranged between 21% and 28%, fine sand between ranged between 21% and 28%, and silt ranged between 7% and 10%. Similarly, Usha Kiranmai, Raja Sekhar, and Hema Latha (2015) reported that the nature of soil textures was characterized by the abundance of silty loam, silty clay, and silty clay loam. Similarly, Pudimadak and Bheemunipatnam wetlands soils are loamy sand in texture.

3.3 pH

The mean pH level was 8.29. Maximum pH values were exhibited in Lagoon area with 8.58 followed by Xaviermunai with 8.51, while minimum was recorded at Koraiyar with 7.9, respectively. Srivibool (2000) suggested that the abundance of Actinobacteria in mangrove soil may actually be due to better quality of the sediments in terms of structure and pH, and Prabhu, Rajkumar, and Perumal (2008) reported pH in the range of 7.2–8.2 in case of Pichavaram mangroves.

3.4 Electrical conductivity and cation exchange capacity

Maximum electrical conductivity and cation exchange capacity were 2.48 dS/m and 23.4 C. mol Proton^{+}/kg recorded in Xaviermunai area compared to other area. It is discussed in our findings, reports of Kathiresan (2000) on Pichavaram mangroves, Kumar and Kumara (2011) on Kundapur mangroves, Vilas and Ghosal (2015) on Sunderbans mangrove.

3.5 Organic carbon and organic matter

The maximum organic carbon values were exhibited in Lagoon area with 0.41% and organic matter as 0.72% was determined. Choudhury (1962) mentioned that the organic matters in mangrove soil belong over 5%. Due to more decomposition of plant and animal residues in mangrove areas, percentage of organic matter is higher than other soils.

3.6 Macronutrients

The macronutrients such as nitrogen, phosphorus, potassium, calcium, magnesium, and sodium were determined in the mangrove soil sample. The average level of macronutrients recorded in Koraiyar area soil sample as 11.05, 7.22, 10.2, 12.3, 11.0, and 5.32 mg/kg of nitrogen, phosphorus, potassium, calcium, and magnesium followed as Lagoon area (9.20, 6.52, 2.99, 12.0, 10.3, and 4.36 mg/kg), Manakkattadu area soil sample (9.33, 6.24, 1.56, 13.6, 9.37, and 2.20 mg/kg), and Xaviermunai area soil sample (0.79, 6.03, 1.12, 11.0, 9.02, and 5.21 mg/kg), respectively. Manikandan and Vijayakumar (2016) found that the maximum content of macronutrients like organic carbon (41%), nitrogen (98.4 kg/ha), phosphorus (7.51 kg/ha), and potassium (887 kg/ha) were recorded at Muthupet mangrove soil, respectively.

3.7 Micronutrients

The micronutrients were estimated in zinc, copper, iron, and manganese content. The average level of micronutrients were recorded in Lagoon area as 1.27, 0.53, 9.22, and 3.55 ppm of zinc, copper, iron, and manganese content compared to other area. The micronutrients like iron (11.2 ppm), manganese (15.2 ppm), zinc (1.30 ppm), and copper (2.52 ppm) were present. The recently investigated were recorded maximum at Manora, Adirampattinam, Kodiyakarai, and Vedaranyam soil samples (Vaijayanthi & Vijayakumar, 2014).

The correlation coefficient analysis of physiochemical parameters versus population density of Actinobacteria was performed. According to the statistics, the significant results were generated and tabulated with Table 21.3. Interestingly, the correlation coefficient analysis between the physical and chemical boundaries of the marine soil samples and total Actinobacterial isolates was performed (Manikandan & Vijayakumar, 2015).

3.8 Isolation and identification of Actinobacteria from the mangrove soil samples

Isolation of Actinobacteria from soil samples of Muthupet mangrove was performed. Totally 399 Actinobacteria were isolated from four different sites and different dilutions. Out of 399, 27 individual genera were observed in four different sites. The maximum number of colonies (120 CFU/mL) and genera (12) were recorded in Lagoon area and least number of colonies (76 CFU/mL) and genera (3) were observed in Manakkattai area (Table 21.2 and Fig. 21.3). The most of the Actinobacteria are white color in aerial spore mass and white or gray color in reverse side was examined. These Actinobacteria were characterized by biochemical studies (Tables 21.3 and 21.4). In a previous study, Sun, Li, and Cai (2017), Minotto,

TABLE 21.2 Isolation and identification of Actinobacteria from Muthupet mangrove soil samples.

Soil collection areas	Number of CFU/g			Total CFU/g	Isolates
	Dilution 10^{-2}	Dilution 10^{-3}	Dilution 10^{-4}		
Koraiyar	42	38	29	112	7
Manakkattai	30	27	19	76	3
Lagoon	46	43	31	120	12
Xaviermunai	35	32	24	91	5
Total (CFU/g)	**153**	**140**	**103**	**399**	**27**

TABLE 21.3 Correlation coefficient analysis of physicochemical parameters versus population density.

	Clay	Slit	Fs	Cs	pH	Ec	CEC	OC	OM	N	P	K	Ca	Mg	Na	Zn	Cu	Fe	Mn	PD
Clay	1																			
Slit	0.99	1																		
Fs	−0.30	−0.95	1																	
Cs	−0.91	0.42	−0.13	1																
pH	−0.17	0.99	−0.89	0.57	1															
Ec	−0.53	0.85	−0.65	0.84	0.93	1														
CEC	0.26	0.96	−1.00	0.16	0.90	0.68	1													
OC	0.81	0.59	−0.81	−0.48	0.45	0.08	0.78	1												
OM	0.89	0.46	−0.70	−0.61	0.30	−0.08	0.68	0.99	1											
N	0.98	−0.20	−0.10	−0.97	−0.37	−0.69	0.06	0.67	0.78	1										
P	0.91	0.40	−0.66	−0.66	0.24	−0.14	0.63	0.98	1.00	0.81	1									
K	0.81	0.59	−0.80	−0.49	0.44	0.07	0.78	1.00	0.99	0.67	0.98	1								
Ca	0.72	−0.69	0.44	−0.94	−0.81	−0.97	−0.48	0.17	0.32	0.85	0.38	0.17	1							
Mg	0.83	0.56	−0.78	−0.52	0.40	0.03	0.76	1.00	0.99	0.70	0.98	1.00	0.21	1						
Na	−0.57	0.82	−0.61	0.86	0.91	1.00	0.64	0.02	−0.13	−0.73	−0.19	0.02	−0.98	−0.02	1					
Zn	−0.98	−0.19	0.48	0.81	−0.02	0.36	−0.44	−0.90	−0.96	−0.92	−0.97	−0.90	−0.58	−0.92	0.41	1				
Cu	−0.53	−0.85	0.97	0.12	−0.74	−0.44	−0.96	−0.93	−0.86	−0.35	−0.83	−0.93	0.21	−0.91	−0.39	0.68	1			
Fe	0.85	0.53	−0.76	−0.55	0.37	0.00	0.73	1.00	1.00	0.72	0.99	1.00	0.24	1.00	−0.05	−0.93	−0.90	1		
Mn	0.97	0.23	−0.52	−0.78	0.06	−0.31	0.48	0.92	0.97	0.90	0.98	0.92	0.54	0.94	−0.37	−1.00	−0.71	0.95	1	
PD	0.36	0.93	−1.00	0.06	0.86	0.60	0.99	0.85	0.75	0.17	0.71	0.84	−0.38	0.82	0.55	−0.53	−0.98	0.80	0.57	1

Ca, Calcium; *CEC*, cation exchange capacity; *Cs*, core sand; *Cu*, copper; *Ec*, electrical conductivity; *Fe*, iron; *Fs*, Find Sand; *K*, potassium; *Ma*, manganese; *Mg*, magnesium; *N*, nitrogen; *Na*, sodium; *OC*, organic carbon; *OM*, organic matter; *P*, phosphorus; *Zn*, zinc.

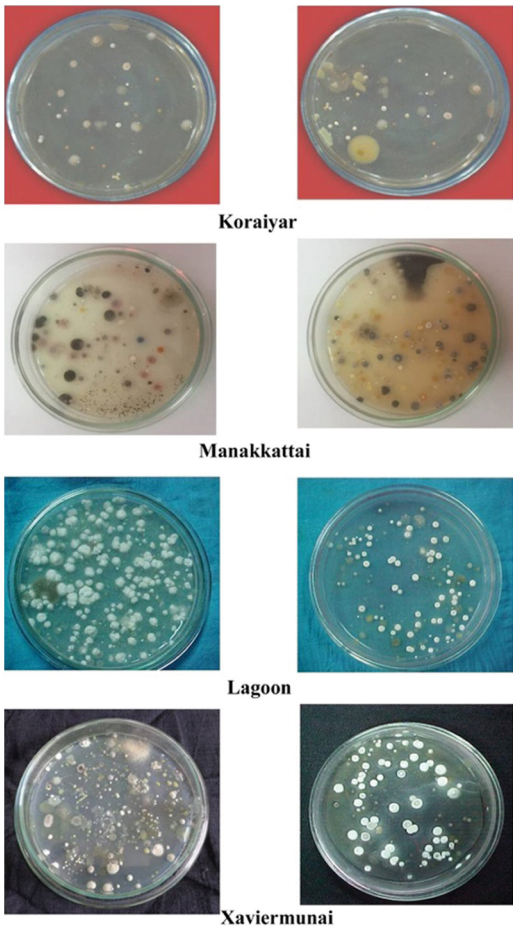


FIGURE 21.3 Isolation of Actinobacteria.

TABLE 21.4 Morphological characteristics of Actinobacteria on SCA medium.

Places	Name of the isolates	Color of aerial spore mass	Color of reverse side	Diffusible pigments
Koraiyar	AVVM01	White	Gray	–
	AVVM02	Light white	Light white	–
	AVVM03	White	White gray	+
Manakkattai	AVVM04	White	Dark gray	–
Lagoon	AVVM05	White	Gray	–
	AVVM06	Gray (white)	Gray	–
	AVVM07	White	Dark gray	–
	AVVM08	White	White gray	–
	AVVM09	White	Dark gray	+
Xaviermunai	AVVM10	Gray	Dark gray	–
	AVVM11	White	White gray	–

+, Soluble pigment; –, in soluble pigment.

Luciana Pasqualini, Martha Trindade, and Teresinha (2014), Baskaran, Vijayakumar, and Mohan (2011), and Singh et al., 2012 were reported, the Actinobacteria isolated from different sources of mangrove environment are identified based on Bergey's Manual of Determinative Bacteriology.

3.9 Screening of antimicrobial activity against human pathogen

The screening of antibacterial activity of Actinobacteria strain (AVVM1–AVVM11) against *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. typhi* were determined by using agar well diffusion method. The maximum zone of inhibition was observed in *Pseudonocardia* sp. (AVVM03) as 19.2 ± 0.26, 18.6 ± 0.37, 20.6 ± 0.20, and 25.3 ± 0.25 (*E. coli*, *P. aeruginosa*, *S. aureus*, and *S. typhi*), and the minimum zone of inhibition was recorded in SVVA4 and SVVA13, respectively (Table 21.5 and Plate 4). The present study supported (Veeramani et al., 2019); cross-streak method was performed, and the minimum inhibition concentration values of isolates against pathogens were calculated. The test pathogens *E. coli*, *S. aureus*, *Klebsiella pneumoniae*, and *P. aeruginosa* were investigated.

According to antifungal activity, the *Pseudonocardia* sp. was more resistant against *A. terreus*, *A. flavus*, *A. fumigatus*, and *Penicillium* sp. as 21.3 ± 1.52, 11.0 ± 0.32, 14.4 ± 0.28, and 14.6 ± 0.20 mm zone of inhibition were determined (Table 21.6). Tanvir, Sajid, Hasnain, Kulik, and Grond (2016) studied antifungal activity of rare actinomycetes, which has been highlighted during the tests carried out on the clinical isolates. Among the tested Actinobacteria, the isolate (*Nocardia*) and the two *Micromonospora* strains were significantly effective against the tested pathogenic yeasts.

3.10 Shrimp pathogen activity

The secondary screening of shrimp pathogens like *V. alginolyticus*, *V. parahaemolyticus*, *V. harveyi*, and *V. vulnificus* against Actinobacteria was performed. The maximum zone of inhibition was recorded in AVVM03 strain and different concentration of 25, 50, 75, and 100 µL as 15.5 ± 0.20, 15.5 ± 0.30, 15.7 ± 0.15, 17.5 ± 0.25 mm in *V. alginolyticus*; 16.4 ± 0.36, 17.2 ± 0.32, 18.3 ± 0.15, and 20.6 ± 0.26 mm in *V. parahaemolyticus*; 22.5 ± 0.25, 23.5 ± 0.30, 24.2 ± 0.30, 25.7 ± 0.15 mm in *V. harveyi* and 18.4 ± 0.36, 19.6 ± 0.15, 20.5 ± 0.25, and 23.2 ± 0.25 mm maximum zone of inhibition showed than compared to other strains, respectively (Table 21.7).

Accordingly, it was reported that one of the marine *Acinetobacter calcoaceticus* showed significant inhibition zones against some fish pathogens such as *Aeromonas hydrophila*, *V. alginolyticus*, and *V. parahaemolyticus* and lower activity against *V. harveyi* and *Pseudomonas fluorescens* shrimp pathogen. Pandey, Ghimire, and Agrawal (2004) have reported that a broad spectrum of antibacterial *Streptomyces* species effective against both Gram-positive and Gram-negative bacteria were isolated from Lobuche area and Lukla area in Khumbu region of Nepal. Antibacterial activity of culture filtrate

TABLE 21.5 Biochemical characterization of Actinobacteria.

	GS	I	MR	VP	Ci	H_2S	U	Ca	O	MP	CHO fermentation test				
Strains no.											G	L	S	M	X
AVVM01	+	–	+	–	+	–	+	+	–	–	+	+	–	–	–
AVVM02	+	–	+	–	–	–	+	+	+	+	+	+	+	+	–
AVVM03	+	–	–	–	–	–	+	++	–	–	+	+	–	+	–
AVVM04	+	–	+	+	+	–	–	–	+	+	+	–	+	+	+
AVVM05	+	–	+	+	+	–	–	–	+	+	+	–	+	+	+
AVVM06	+	–	–	–	+	+	+	+	+	D	+	+	D	+	+
AVVM07	+	–	–	–	+	+	+	+	+	D	+	+	D	+	+
AVVM08	+	–	–	–	+	+	+	+	+	D	+	+	D	+	+
AVVM09	+	–	–	–	+	+	+	+	+	D	+	+	D	+	+
AVVM10	+	–	–	+	–	+	+	+	+	–	+	+	–	+	–
AVVM11	+	–	+	–	–	+	–	+	+	D	+	+	–	+	–

Ca, Catalase; *Ci*, citrate; *D*, different strain; *G*, glucose; *GS*, gram stain; *I*, indole; *L*, lactose; *M*, maltose; *MP*, melanin production; *MR*, methyl red; *O*, oxidase; *S*, sucrose; *U*, urease; *VP*, voges proskauer; *X*, xylose.

TABLE 21.6 Identification of Actinobacteria.

S. no.	Strain no.	Identified as
1	AVVM01	*Jonesia* sp.
2	AVVM02	*Kineospora* sp.
3	AVVM03	*Pseudonocardia* sp.
4	AVVM04	*Saccharopolyspora* sp.
5	AVVM05	*Saccharopolyspora* sp.
6	AVVM06	*Streptomyces* sp.
7	AVVM07	*Streptomyces* sp.
8	AVVM08	*Streptomyces* sp.
9	AVVM09	*Streptomyces* sp.
10	AVVM10	*Nocardia* sp.
11	AVVM11	*Terrabacter* sp.

TABLE 21.7 Screening of antibacterial activity of Actinobacterial isolates against human pathogen.

	Zone of inhibition (mm)			
Isolates strain	*Escherichia coli* (MTCC1687)	*Pseudomonas aeruginosa* (MTCC424)	*Staphylococcus aureus* (MTCC737)	*Salmonella typhi* (MTCC98)
Jonesia sp.	16.6 ± 0.32	15.5 ± 0.30	18.3 ± 0.17	17.4 ± 0.26
Kineospora sp.	16.3 ± 0.37	14.5 ± 0.36	17.2 ± 0.30	16.6 ± 0.26
Pseudonocardia sp.	19.6 ± 0.26	18.6 ± 0.37	20.6 ± 0.20	25.3 ± 0.25
Saccharopolyspora sp.	15.6 ± 0.37	14.4 ± 0.36	16.5 ± 0.30	16.4 ± 0.35
Saccharopolyspora sp.	11.2 ± 0.12	6.10 ± 0.26	9.10 ± 0.29	11.4 ± 0.30
Streptomyces sp.	17.3 ± 0.26	15.6 ± 0.32	18.4 ± 0.15	17.5 ± 0.23
Streptomyces sp.	10.6 ± 0.23	6.25 ± 0.13	9.89 ± 0.33	9.5 ± 0.20
Streptomyces sp.	14.5 ± 0.47	12.6 ± 0.32	15.5 ± 0.30	15.4 ± 0.30
Streptomyces sp.	18.4 ± 0.35	15.6 ± 0.32	20.4 ± 0.35	23.6 ± 0.15
Nocardia sp.	11.6 ± 0.26	9.70 ± 0.15	-	-
Terrabacter sp.	14.3 ± 0.26	10.4 ± 0.37	13.4 ± 0.25	13.4 ± 0.15

Standard deviation ± standard error.

obtained from *Streptomyces* sp. No. 87 against Gram-positive and Gram-negative bacteria was reported (Charoensopharat, Thummabenjapone, Sirithorn, & Thammasirirak, 2008).

3.11 Antibiotic sensitivity test

The antibiotic susceptibility testing was performed by the disc diffusion method. The commercial antibiotics like chloramphenicol, ciprofloxacin, gentamycin, and polymyxin were 14.0 ± 4.66, 14.0 ± 4.66, 11.6 ± 3.86, and 7.33 ± 2.43-mm zone of inhibition recorded against *V. alginolyticus*, respectively, whereas *V. parahaemolyticus* was 11.6 ± 3.86, 5.00 ± 1.66, 7.66 ± 2.55, 11.3 ± 3.76, and 7.33 ± 2.43-mm zone of inhibition with chloramphenicol, gentamycin, polymyxin, streptomycin, and rifampicin recorded, respectively. In the case of commercial antibiotics like ciprofloxacin, gentamycin, polymyxin, streptomycin, and rifampicin, it was 21.3 ± 7.10, 12.3 ± 4.10, 11.6 ± 3.86, 6.00 ± 2.00, and 7.33 ± 2.43-mm zone of inhibition against *V. vulnificus* was suppressed in the antibiotics like chloramphenicol, ciprofloxacin, erythromycin, gentamycin, polymyxin, streptomycin, and rifampicin was 11.3 ± 3.76, 7.00 ± 2.33, 6.00 ± 2.00, 12.0 ± 4.12, 7.33 ± 2.43, 14.0 ± 4.66, and 14.3 ± 4.66-mm zone of inhibition analyzed against pathogen, respectively (Table 21.8).

TABLE 21.8 Screening of antifungal activity of Actinobacterial isolates against human pathogen.

Isolates strain	Zone of inhibition (mm)			
	Aspergillus flavus (MTCC9606)	*Aspergillus terreus* (MTCC2584)	*Aspergillus fumigatus* (MTCC1782)	*Penicillium* sp. (MTCC5108)
Jonesia sp.	16.4 ± 0.30	8.90 ± 0.40	10.7 ± 0.20	10.4 ± 0.26
Kineospora sp.	15.6 ± 0.37	8.06 ± 0.32	10.7 ± 0.20	9.90 ± 0.26
Pseudonocardia sp.	21.3 ± 1.52	11.0 ± 0.32	14.4 ± 0.28	14.6 ± 0.20
Saccharopolyspora sp.	14.5 ± 0.32	8.07 ± 0.20	7.40 ± 0.21	9.20 ± 0.26
Saccharopolyspora sp.	2.80 ± 0.76	3.20 ± 0.37	4.00 ± 0.20	2.10 ± 0.35
Streptomyces sp.	–	–	4.26 ± 0.64	3.00 ± 0.15
Streptomyces sp.	18.4 ± 0.15	10.1 ± 0.55	10.7 ± 0.52	11.5 ± 0.31
Streptomyces sp.	3.60 ± 0.20	4.04 ± 0.36	3.40 ± 0.35	2.80 ± 0.30
Streptomyces sp.	19.5 ± 0.25	10.4 ± 0.35	11.5 ± 0.35	7.30 ± 0.15
Nocardia sp.	10.2 ± 0.41	4.04 ± 0.32	–	3.03 ± 0.25
Terrabacter sp.	11.4 ± 0.45	5.60 ± 0.26	5.05 ± 0.25	8.06 ± 0.20

Some researchers (Kelch & Lee, 1978; Beaucage & Fox, 1979; Amaro et al., 1990; Morinigo et al., 1990; Muraleedharan, Kannapiran, KalaiGandhi, & Michale, 2009) detailed about the shrimp pathogens *Vibrio alcaligenes*, *V. alginolyticus*, *Vibrio cholerae*, *V. parahaemolyticus*, and *V. vulnificus* showing resistance against erythromycin, ampicillin, and penicillin G antibiotics. Deepika and Kannabiran (2010) reported that the *K. pneumoniae* produces an inhibition zone of 15 mm compared with standard drug penicillin G (10 µg/disc). No activity was seen against *E. coli* and *P. aeruginosa*. Antifungal activity was observed against *A. flavus* (10 mm) and *Aspergillus niger* (25 mm) when compared to standard drug nystatin (100 µg/disc) that produced a zone of clearance of 16–20 mm, respectively. Actinomycetes are the most biotechnologically valuable prokaryotes responsible for the production of about half of the discovered bioactive secondary metabolites including antibiotics (Yuan et al., 2010).

3.12 Molecular characterization of Actinobacterial strain

The amplification of 16S rRNA gene of the *P. alaniniphila* (AVVM03) was deposited in GenBank (NCBI, United States) under the accession no. MN558974. The *P. alaniniphila* strain constructed for phylogenetic analysis by neighbor-joining tree based on 16S rRNA gene sequences showed that the isolate shares a same clade with *P. alaniniphila* and occupies a distinct phylogenetic position within the radiation including representatives of the family *Pseudonocardia.* The secondary structure prediction of *P. alaniniphila* was observed in stem-74, and major loop-4 and minor loop-71 were recorded, respectively (Figs. 21.4 and 21.5).

Our study compared to the phylogenetic tree from representative and the most similar strains indicated that strain KRG-1 belongs to the genus *Streptomyces* (Charousova, Juraj, Lukas, Miroslava, & Sona, 2019). The BLAST search result showed that VITDDK2 showed 93% similarity to the isolate *Streptomyces* sp. strain 346 (AJ278761). A phylogenetic tree was constructed based on neighbor-joining method and the RNA secondary structure predicted for 16S rRNA gene of *Streptomyces* sp. VITDDK2 showed the free energy of −150.1 kcal/mol, threshold energy of −4.0 with Cluster factor 2, Conserved factor 2, and Compensated factor 4 (Deepika & Kannabiran, 2010).

3.13 Experimental design

The experimental design was conducted at Eripurakarai, Adirampattinam. Five different treatments were adopted, and the growth of *L. vannamei* shrimp was determined. The trail tank was performed and physicochemical properties were analyzed in water from the tank, growth parameters of *L. vannamei*, biochemical and immunological parameters were analyzed in every intervals of the tank. Rengipipat, Rukpratanpom, Piyatiratitivorakul, and Menasaveta (2000) reported greater immunity indices (THC, phagocytic activity, phenol oxidase activity, and serum antibacterial activity) in *Penaeus monodon*

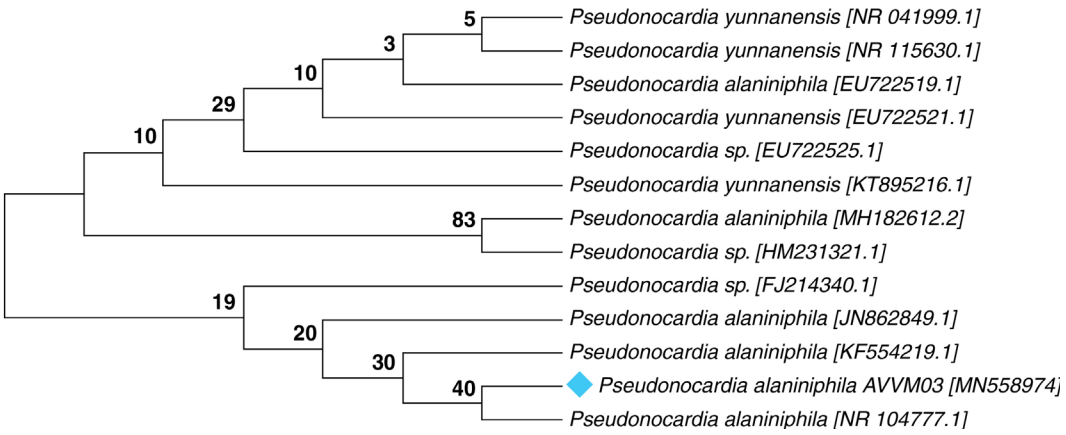


FIGURE 21.4 Phylogenetic relationship of *Pseudonocardia alaniniphila*.

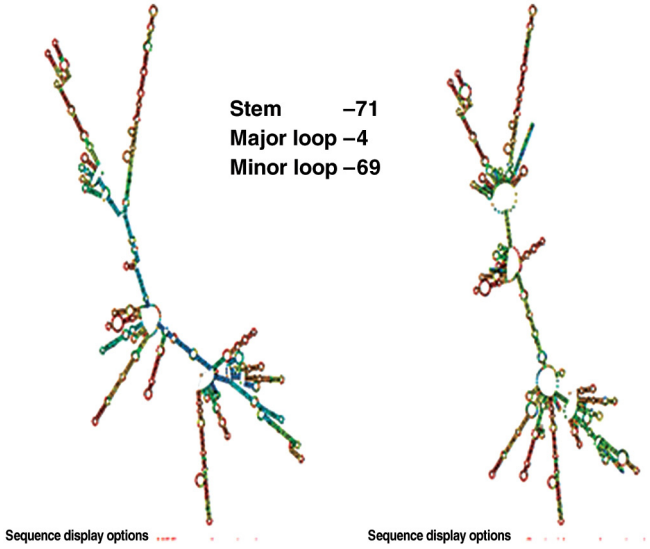


FIGURE 21.5 Secondary prediction of *Pseudonocardia alaniniphila*.

fed with live bacterium *Bacillus* SII. *Bacillus* SII surface antigens or their metabolites might have acted as immunogens for shrimp immune defense.

Initially physiochemical parameters like color, temperature, pH, turbidity, salinity, alkalinity, total hardness, dissolved oxygen, biochemical oxygen demand, chemical oxygen demand, calcium, nitrate, nitrite, iron, and ammonia were analyzed for 50, 75, and 100 days of water samples. The highest physicochemical parameters recorded in Tank-4 were treated with *P. alaniniphila* + β-glucon has also maintained in 75 and 100 days of intervals (Table 21.9). The pH of 7.4–9.7 in shrimp culture pond area was within safe range for aquatic life (Tanner, Craggs, Sukias, & Park, 2005). The pH concentration gets changed with time due to the changes in temperature, salinity, and biological activity. Most of the natural waters are generally alkaline due to the presence of sufficient quantities of carbonate (Sachidanandamurthy & Yajurvedi, 2006).

The morphometric analysis of *L. vannamei* culture tank for the different period with various parameters like length, weight, weight grain, survival, and feed conversion rate was determined. The maximum growth characters were recorded in Tank-3 (*P. alaniniphila*+ Vit. C) as 6.9 mm, 7.3 g, 1.7 g, 100%, 1:1 of length, weight, weight gain, survival, and FCR on 50th day, in 75th day as Tank-4 (*P. alaniniphila* + β-glucan) was 13.8, 15.4, 7.7 g, 91%, 1%:2.5% of length, weight, weight gain, survival, and FCR and 100 days was estimated on length (15.8 g), weight (21.8 g), weight gain (7.7 g), survival (75%), and FCR (1:2.5) recorded, respectively (Table 21.10). Similarly when yeast *Saccharomyces cerevisiae* is used, Nates, 1994

TABLE 21.9 Secondary screening of selected Actinobacteria against shrimp pathogens by well diffusion method.

Potential strain	Different concentration (μL)	Zone of inhibition (mm)			
		Vibrio alginolyticus (MTCC4442)	*Vibrio parahaemolyticus* (MTCC451)	*Vibrio harveyi* (MTCC3438)	*Vibrio vulnificus* (MTCC1145)
Jonesia sp.	25	–	–	–	–
	50	–	–	–	6.20 ± 0.25
	75	–	–	–	9.03 ± 0.20
	100	–	–	–	9.04 ± 0.20
Kineospora sp.	25	–	–	–	9.60 ± 0.20
	50	–	14.2 ± 0.26	11.6 ± 0.25	10.3 ± 0.32
	75	13.2 ± 0.25	–	–	10.5 ± 0.17
	100	13.2 ± 0.25	–	6.03 ± 0.15	10.7 ± 0.20
Pseudonocardia sp.	25	15.5 ± 0.20	16.4 ± 0.36	22.5 ± 0.25	18.4 ± 0.36
	50	15.5 ± 0.30	17.2 ± 0.32	23.5 ± 0.30	19.6 ± 0.15
	75	15.7 ± 0.15	18.3 ± 0.15	24.2 ± 0.23	20.5 ± 0.25
	100	17.5 ± 0.25	20.6 ± 0.26	25.7 ± 0.15	23.2 ± 0.25
Saccharopolyspora sp.	25	–	13.6 ± 0.15	9.04 ± 0.12	–
	50	13.2 ± 0.20	14.2 ± 0.25	9.03 ± 0.15	11.4 ± 0.17
	75	–	–	–	–
	100	–	13.6 ± 0.15	8.04 ± 0.25	11.5 ± 0.21
Saccharopolyspora sp.	25	–	–	–	–
	50	6.20 ± 0.30	–	–	11.5 ± 0.30
	75	–	–	–	–
	100	8.05 ± 0.15	–	–	11.7 ± 0.20
Streptomyces sp.	25	13.2 ± 0.26	14.5 ± 0.05	17.3 ± 0.20	16.0 ± 0.26
	50	13.2 ± 0.26	14.4 ± 0.20	17.4 ± 0.15	16.4 ± 0.11
	75	13.4 ± 0.15	14.5 ± 0.25	17.5 ± 0.30	16.7 ± 0.15
	100	13.6 ± 0.15	15.2 ± 0.30	17.5 ± 0.30	16.8 ± 0.15
Streptomyces sp.	25	8.03 ± 0.23	–	–	–
	50	10.1 ± 0.15	–	11.6 ± 0.15	–
	75	–	–	–	–
	100	12.5 ± 0.11	–	10.3 ± 0.32	12.5 ± 0.10
Streptomyces sp.	25	11.3 ± 0.20	5.50 ± 0.30	10.6 ± 0.20	–
	50	11.4 ± 0.17	–	–	–
	75	12.2 ± 0.26	–	11.2 ± 0.05	–
	100	12.3 ± 0.20	6.70 ± 0.20	11.5 ± 0.10	–
Streptomyces sp.	25	14.2 ± 0.26	15.4 ± 0.15	18.6 ± 0.11	17.1 ± 0.32
	50	14.4 ± 0.17	15.6 ± 0.17	19.3 ± 0.28	17.5 ± 0.30
	75	14.6 ± 0.15	15.5 ± 0.30	19.6 ± 0.28	17.7 ± 0.20
	100	14.9 ± 0.17	16.1 ± 0.20	20.4 ± 0.05	18.5 ± 0.21
Nocardia sp.	25	10.7 ± 0.20	–	7.03 ± 0.23	14.5 ± 0.20
	50	11.2 ± 0.26	–	–	15.5 ± 0.26
	75	–	–	15.9 ± 0.15	15.5 ± 0.30
	100	10.2 ± 0.26	–	16.2 ± 0.26	15.6 ± 0.20
Terrabacter sp.	25	10.3 ± 0.17	–	16.3 ± 0.20	–
	50	10.3 ± 0.20	–	16.5 ± 0.25	–
	75	–	–	16.7 ± 0.26	–
	100	10.2 ± 0.26	–	17.3 ± 0.17	–

Standard deviation ± standard error.

TABLE 21.10 Effect of antibiotic sensitivity assay by screening against shrimp pathogens.

Name of the antibiotics	Zone of inhibition (mm)			
	Vibrio alginolyticus (MTCC4442)	*Vibrio parahaemolyticus* (MTCC451)	*Vibrio harveyi* (MTCC3438)	*Vibrio vulnificus* (MTCC1145)
A^{25}	–	–	–	–
C^{30}	14.0 ± 4.66	11.6 ± 3.86	–	11.3 ± 3.76
CIP^{5}	14.0 ± 4.66		21.3 ± 7.10	7.00 ± 2.33
E^{5}	–	–	–	6.00 ± 2.00
GEN^{10}	11.6 ± 3.86	5.00 ± 1.66	12.3 ± 4.10	12.0 ± 4.12
P^{10}	–	–	–	–
PB^{300}	7.33 ± 2.43	7.66 ± 2.55	11.6 ± 3.86	7.33 ± 2.43
S^{25}	–	11.3 ± 3.76	6.00 ± 2.00	14.0 ± 4.66
RIF^{5}	–	7.33 ± 2.43	7.33 ± 2.43	14.3 ± 4.66

A^{25}, Ampicillin; *C^{30}*, chloramphenicol; *CIP^{5}*, ciprofloxacin; *E^{5}*, erythromycin; *GEN^{10}*, gentamycin; *P^{10}*, penicillin; *PB^{300}*, polymyxin; *RIF^{5}*, rifampicin; *S^{25}*, streptomycin.

and Devaraja, Yusoff, and Shariff (2002) noticed higher SRs in treatment ponds compared to control in *P. monodon* and *L. vannamei* grew outs, respectively.

The biochemical parameters of protein, CHOs, lipid, moisture, ash, calcium, magnesium, sodium, potassium, phosphorus, manganese, and iron in every interval of shrimp culture *L. vannamei* were determined. The huge quantity of protein, calcium, sodium, and potassium was 121.5% ± 0.21%, 155.6 ± 0.36, 65.61 ± 0.11, and 59.68 ± 0.05 mg/g in 50th day, 123.6 ± 0.20, 76.4 ± 0.11, 156.8 ± 0.17, 69.37 ± 0.25, and 60.83 ± 0.15 mg/g, in 75th day and 129.8 ± 0.20 mg/g, 8.2% ± 0.12%, 2.3% ± 0.30%, 75.7% ± 0.11%, 2.4% ± 0.15%, 158.5 ± 0.25, 17.77 ± 0.18, 69.84 ± 0.17, 62.85 ± 0.13, 7.15 ± 0.06, 0.97 ± 0.02, and 6.15 ± 0.03 mg/g in 100 days were recorded excellent and significant results observed in Tank-4 (*P. alaniniphila* + β-glucan) than compared to other tank with control tank respectively (Table 21.11). At the same time, Mohanta, Mohanty, Jena, and Sahu (2007) reported that the protein level of aquaculture animals initially increased then decreased with increasing dietary protein levels.

Immunological study were analyzed in *L. vannamei* culture Tank-4 has β-glucan + *P. alaniniphila* has maximum coagulase activity and THC (161.5 ± 0.20, 174.2 ± 0.15 and 158.3 ± 0.23 and 39.7 ± 0.15, 38.8 ± 0.15 and 39.4 ± 0.17 × 10^6 cells/mL) recorded respectively. The intracellular superoxide anion activity of *L. vannamei* detected was after 50, 75, and 100 days of culture. In Tank-4, the highest recorded superoxide anion was 2.2% ± 0.1%, 2.5% ± 0.3%, and 2.9% ± 0.5% and prophenoloxidase activity (23.0% ± 0.9%, 27.0% ± 0.6%, and 30.0% ± 0.9%) was every interval of 50, 75, and 100 days (Table 21.12). A similar result, the THC and O_2 production also started recovering after 6 days post challenge. proPO and O_2^- levels was obtained for the herbal extracts treated to *P. monodon* (Chang, Su, Yung, & Liao, 2003).

4 Conclusion

From this study, it can be concluded that mangrove Actinobacteria strains can protect the aquaculture industry. Some of these physicochemical parameters are influencing the accumulation of Actinobacterial growth in environment. Among the 11 Actinobacterial strains, *P. alaniniphila* (AVVM03) has extraordinary biological activity against human as well as shrimp pathogens. The Tank-3 trail was highlighted that preparative for enhancing production of biochemical content and growth parameter also were performed in *L. vannamei*, because the perspective bioactive molecules were estimated in the probiotics. The probiotics are used for the nutraceutical products and have excellent performance for aquaculture industry (Tables 21.13 and 21.14).

TABLE 21.11 Analysis of water physiochemical parameters of *Litopenaeus vannamei* culture tank.

Name of the Parameter	50th Day					75th Day					100th Day				
	Tank-1	Tank-2	Tank-3	Tank-4	Tank-5	Tank-1	Tank-2	Tank-3	Tank-4	Tank-5	Tank-1	Tank-2	Tank-3	Tank-4	Tank-5
Color	White transparent color					Slight greenish color with transparent					White transparent color				
Temperature (°C)	29.8	29.4	30.1	31.1	30.4	30.7	30.2	30.4	30.3	30.8	31	31.4	30.9	31.3	31.8
pH	7.5	7.6	7.9	8	8.1	7.6	7.68	7.73	7.9	7.65	8	8.15	8.2	8.3	8.4
Turbidity (%)	49.3	43.4	44.6	44.2	42.6	47.2	48.1	48.3	44.6	48.7	43	44.3	42.6	41.6	42.3
Salinity (ppt)	29	30	31	32	32	30.2	30.5	31.2	31.6	31.5	31.3	32.3	32.4	32.6	32.6
Alkalinity (mg/L)	148	160	152	164	160	178	162	176	152	164	180	196	210	204	214
Total hardness (ppm)	1800	1640	1750	1680	1660	1650	2200	2340	1750	2290	1740	2160	2450	2350	2260
DO (mg/L)	5.4	5.7	5.6	5.2	5.2	5.2	5.4	5	5.6	5.45	5.2	4.9	4.3	4.5	4.6
BOD (mg/L)	12.6	14.5	16.4	14.3	16.2	14.5	8.6	20	16.4	8.64	22.6	18.5	30.2	29.6	31.5
COD (mg/L)	22.1	22	22.3	20.2	19.2	27.3	28.6	29.3	22.3	26.6	29	31	34	32	36
Calcium (mg/L)	40	65	74	85	75	140	165	135	74	171	160	155	145	156	171
Nitrate (mg/L)	160	185	210	320	190	0.04	0.07	0.08	0.03	0.08	0.06	0.01	0.13	0.02	0.06
Nitrite (mg/L)	0.02	0.01	0.03	0.02	0.01	0.06	0.1	0.12	0.02	0.15	0.02	0.027	0.033	0.035	0.25
Iron (mg/L)	0.01	0.01	0.02	0.02	0.01	0.02	0.025	0.027	0.03	0.026	0.015	0.027	0.039	0.032	0.037
Ammonia (mg/L)	0.01	0.01	0.03	0.02	0.01	0.01	0.02	0.03	0.01	0.02	0.015	0.027	0.031	0.02	0.03

Tank-1, Control; *Tank-2*, *P. alaniniphila*; *Tank-3*, *P. alaniniphila* + Vit. C; *Tank-4*, *P. alaniniphila* + β-glucan; *Tank-5*, *P. alaniniphila* + FOS.

TABLE 21.12 Growth characteristics of *Litopenaeus vannamei* culture tank for the periods.

50 Days					
Name of the parameters	**Tank-1**	**Tank-2**	**Tank-3**	**Tank-4**	**Tank-5**
Length (mm)	6.8	6.8	6.9	6.3	6.4
Weight (g)	7.5	7.1	7.3	7.0	7.2
Weight gain (g)	1.3	1.5	1.7	1.4	1.5
Survival (%)	100	100	100	100	100
Feed conversion ratio	1:1	1:1	1:1	1:1	1:1
75 Days					
Length (mm)	10.1	11.9	13.6	13.8	13.2
Weight (g)	10.2	14.1	14.5	15.4	15.2
Weight gain (g)	2.6	8.6	6.6	7.7	7.3
Survival (%)	86	90	90.66	91	92
Feed conversion ratio	1:2.27	1:2.5	1:2.5	1:2.5	1:2.5
100 Days					
Length (mm)	13.5	14.5	14.9	15.3	15.8
Weight (g)	13.4	18.4	21.3	21.8	21.6
Weight gain (g)	3.4	5.9	5.1	7.7	6.8
Survival (%)	68	70	74	75	72
Feed conversion ratio	1:2.5	1:2.4	1:2.5	1:2.5	1:2.5

β-Glucan, Beta glucan; *FOS*, fructooligosaccharides; *Tank-1*, control; *Tank-2*, *P. alaniniphila*; *Tank-3*, *P. alaniniphila* + Vit. C; *Tank-4*, *P. alaniniphila* + β-glucan; *Tank-5*, *P. alaniniphila* + FOS; *VIT-C*, vitamin C.

TABLE 21.13 Biochemical changes in the tissue of *Litopenaeus vannamei* fed with growth promoter incorporated diets after 50 days of culture.

Biochemical Parameters	50th Day					75th Day					100th Day				
	Tank-1	Tank-2	Tank-3	Tank-4	Tank-5	Tank-1	Tank-2	Tank-3	Tank-4	Tank-5	Tank-1	Tank-2	Tank-3	Tank-4	Tank-5
Protein (%)	90.5 ± 0.08	110.2 ± 0.05	112.0 ± 0.10	111.1 ± 0.10	105.4 ± 0.08	110.0 ± 0.25	115.1 ± 0.10	109.8 ± 0.20	120.3 ± 0.18	122.1 ± 0.10	111.8 ± 0.28	116.6 ± 0.15	110.8 ± 0.30	121.5 ± 0.21	125.6 ± 0.20
Carbohydrates (%)	2.5 ± 0.10	3.5 ± 0.10	2.5 ± 0.16	5.6 ± 0.09	2.6 ± 0.05	4.1 ± 0.28	6.1 ± 0.29	5.3 ± 0.30	5.5 ± 0.25	5.0 ± 0.10	4.5 ± 0.30	6.5 ± 0.30	5.5 ± 0.36	7.6 ± 0.20	5.6 ± 0.15
Lipid (%)	1.0 ± 0.05	1.21 ± 0.02	1.30 ± 0.17	1.32 ± 0.11	1.38 ± 0.11	1.5 ± 0.10	1.7 ± 0.02	1.8 ± 0.16	1.72 ± 0.10	1.4 ± 0.10	1.7 ± 0.15	1.8 ± 0.03	1.9 ± 0.17	1.73 ± 0.11	1.6 ± 0.11
Moisture (%)	50.2 ± 0.13	60.13 ± 0.25	62.3 ± 0.27	63.7 ± 0.10	63.9 ± 0.17	69.5 ± 0.20	70.3 ± 0.20	74.1 ± 0.30	71.4 ± 0.10	72.1 ± 1.15	70.2 ± 0.23	71.53 ± 0.25	75.3 ± 0.37	72.7 ± 0.20	73.9 ± 1.17
Ash (%)	1.0 ± 0.10	1.0 ± 0.01	1.0 ± 0.01	1.1 ± 0.05	1.2 ± 0.01	1.1 ± 0.25	1.5 ± 0.01	1.1 ± 0.04	1.5 ± 0.11	1.5 ± 0.04	1.4 ± 0.30	1.6 ± 0.01	1.5 ± 0.05	1.9 ± 0.15	1.8 ± 0.05
Calcium (mg/g)	80.1 ± 0.05	82.0 ± 0.05	82.6 ± 0.05	83.3 ± 0.06	84.5 ± 0.05	150.1 ± 0.12	152.1 ± 0.10	150.1 ± 0.20	154.2 ± 0.30	152.1 ± 0.20	151.4 ± 0.15	153.7 ± 0.15	152.6 ± 0.25	155.6 ± 0.36	154.5 ± 0.25
Magnesium (mg/g)	10.12 ± 0.01	12.12 ± 0.02	13.13 ± 0.03	14.11 ± 0.05	15.03 ± 0.07	13.10 ± 0.01	14.00 ± 0.03	14.13 ± 0.04	15.13 ± 0.09	15.13 ± 0.09	14.51 ± 0.01	15.64 ± 0.04	15.23 ± 0.05	16.63 ± 0.10	16.33 ± 0.11
Sodium (mg/g)	55.1 ± 0.12	58.14 ± 0.06	57.18 ± 0.07	55.10 ± 0.09	66.34 ± 0.05	64.6 ± 0.30	67.11 ± 0.15	66.18 ± 0.09	64.11 ± 0.10	65.11 ± 0.08	65.6 ± 0.32	68.34 ± 0.16	67.58 ± 0.10	65.61 ± 0.11	66.34 ± 0.09
Potassium (mg/g)	50.00 ± 0.02	52.15 ± 0.01	53.18 ± 0.02	54.00 ± 0.03	55.14 ± 0.01	55.00 ± 0.03	57.54 ± 0.01	55.18 ± 0.04	56.00 ± 0.07	57.14 ± 0.10	56.35 ± 0.04	58.55 ± 0.01	59.68 ± 0.05	57.70 ± 0.08	58.34 ± 0.11
Phosphorus (mg/g)	5.00 ± 0.03	5.10 ± 0.02	5.14 ± 0.02	5.00 ± 0.03	5.16 ± 0.02	6.19 ± 0.06	6.11 ± 0.05	6.14 ± 0.03	6.00 ± 0.05	6.16 ± 0.03	6.39 ± 0.06	6.81 ± 0.05	6.44 ± 0.03	6.44 ± 0.07	6.96 ± 0.04
Manganese (mg/g)	0.37 ± 0.01	0.46 ± 0.01	0.54 ± 0.01	0.67 ± 0.01	0.53 ± 0.01	0.67 ± 0.02	0.76 ± 0.03	0.74 ± 0.01	0.77 ± 0.01	0.73 ± 0.02	0.77 ± 0.02	0.86 ± 0.03	0.84 ± 0.01	0.87 ± 0.01	0.83 ± 0.02
Iron (mg/g)	4.11 ± 0.1	4.16 ± 0.01	4.10 ± 0.11	4.10 ± 0.06	4.18 ± 0.06	5.32 ± 0.1	5.86 ± 0.02	4.86 ± 0.121	5.40 ± 0.07	5.78 ± 0.11	5.32 ± 0.1	5.86 ± 0.02	4.86 ± 0.121	5.40 ± 0.07	5.78 ± 0.11

Standard deviation ± standard error. *Tank-1*, Control; *Tank-2*, *P. alaniniphila*; *Tank-3*, *P. alaniniphila* + Vit. C; *Tank-4*, *P. alaniniphila* + β-glucan; *Tank-5*, *P. alaniniphila* + FOS.

TABLE 21.14 Immunological activity of *Litopenaeus vannamei* fed with growth promoter incorporated diets after 50 days of culture.

Days	Treatment	Coagulase activity (s)	Total Hemocyte Count (THC) ($\times 10^6$ cells/mL)	Intra cellular superoxide anion	Prophenoloxidase activity
50th Day	Tank-1	285.7 ± 0.15	26.6 ± 0.32	1.2 ± 0.2	14.0 ± 0.2
	Tank-2	243.6 ± 0.20	35.4 ± 0.35	1.4 ± 0.3	16.0 ± 1.1
	Tank-3	221.5 ± 0.30	37.3 ± 0.20	1.8 ± 0.5	18.0 ± 0.9
	Tank-4	161.5 ± 0.20	39.7 ± 0.15	2.2 ± 0.1	23.0 ± 0.9
	Tank-5	180.5 ± 0.25	38.7 ± 0.26	1.9 ± 0.4	19.0 ± 0.5
75th Day	Tank-1	277.2 ± 0.10	25.2 ± 0.20	1.6 ± 0.3	19.0 ± 0.6
	Tank-2	253.5 ± 0.36	34.1 ± 0.36	1.9 ± 0.1	22.0 ± 0.8
	Tank-3	215.6 ± 0.20	36.4 ± 0.25	2.3 ± 0.6	25.0 ± 0.4
	Tank-4	174.2 ± 0.15	38.8 ± 0.15	2.5 ± 0.3	27.0 ± 0.6
	Tank-5	181.6 ± 0.25	37.3 ± 0.17	2.2 ± 0.1	24.0 ± 1.0
100th Day	Tank-1	284.3 ± 0.20	25.4 ± 0.15	2.1 ± 0.2	23.0 ± 1.1
	Tank-2	241.5 ± 0.25	36.4 ± 0.25	2.5 ± 0.6	26.0 ± 0.5
	Tank-3	219.5 ± 0.30	38.5 ± 0.11	2.7 ± 0.4	29.0 ± 0.8
	Tank-4	158.3 ± 0.23	39.4 ± 0.17	2.9 ± 0.5	30.0 ± 0.9
	Tank-5	178.5 ± 0.35	37.6 ± 0.20	2.7 ± 0.3	27.0 ± 1.2

Standard deviation ± standard error.

Acknowledgments

The authors gratefully acknowledge the Principal, A. Veeraiya Vandayar, Sri Pushpam College (Auto), Poondi, Thanjavur, and Indian Biotrack Research Institute, Thanjavur, for providing necessary facilities to carry out the research work.

References

Amaro, C., Toranzo, A. E., Gonzalez, E., Blanco, A., Pujalte, J., Aznar R., M. J., et al. (1990). Surface and virulence properties of environmental *Vibrio cholerae* non-O1 from Albufera Lake (Valencia, Spain). *Applied and Environmental Microbiology*, *53*, 603–605.

AOAC. (1990). In *Official methods of analysis* (15th ed.). Washington, DC: Association of Official Analytical Chemist.

APHA; AWWA; WPCF. (1985). In *Standard methods for the examination of water and waste water* (15). (14th ed., pp. 1–1134). Washington, DC: American Public Health Association.

Baskaran, R., Vijayakumar, R., & Mohan, P. M. (2011). Enrichment method for the isolation of bioactive actinomycetes from mangrove sediments of Andaman Islands, India. *Malaysian Journal of Microbiology*, *7*(1), 26–32.

Bautista, M., Eusebio, P., & Welsh, T. (2003). Utilization of feed pea, *Pisum sativum*, meal as a protein source in practical diets juvenile tiger shrimp, *Penaeus monodon*. *Aquaculture*, *225*, 121–131.

Beaucage, C. M., & Fox, J. G. (1979). Transmissible antibiotic resistance in *Salmonella* isolated Randon-source cats purchased for use in research. *American Journal of Veterinary Research*, *40*, 849–851.

Boonyaratpalin, M. (1993). Nutritional requirements of grouper, *Epinephelus*. In *The proceedings of workshop on grouper research and development* (pp. 50–55). .

Chang, C. F., Su, M. S., Yung, H., & Liao, I. (2003). Dietary /3-1,3 glucan effectively improves immunity and survival of *Penaeus monodon* challenged with white spot syndrome virus. *Fish and Shellfish Immunology*, *15*, 297–310.

Charoensopharat, K., Thummabenjapone, P., Sirithorn, P., & Thammasirirak, S. (2008). Antibacterial substance produced by *Streptomyces* sp. no. 87. *African Journal of Biotechnology*, *7*, 1362–1368.

Charousova, I., Juraj, M., Lukas, H., Miroslava, C., & Sona, J. (2019). Antimicrobial activity of actinomycetes and characterization of actinomycin-producing strain KRG-1 isolated from Karoo, South Africa. *Brazilian Journal of Pharmaceutical Sciences*, *55*, 1–11.

Choudhury, A. M. (1962). *Working plan of the Sundarban forest division for the period from 1960-60 to 1979-80* (3 Vol.). Tejgaon, Dacca: East Pakistan Government Press. Printed 1968.

Das, S., Ward, L. R., & Burke, C. (2008). Prospects of using marine Actinobacteria as probiotics in aquaculture. *Applied Microbiology and Biotechnology*, *81*, 419–429.

Deepika, L., & Kannabiran, K. (2010). Isolation and characterization of antagonistic actinomycetes from marine soil. *Journal of Microbial and Biochemical Technology*, *2*, 001–006.

Deshmukh, M. B., & Sridhar, K. R. (2002). Distribution and Antimicrobial activity of Actinomycetes of a Fresh water coastal stream. *Asian Journal of Microbiology, Biotechnology & Environmental Sciences*, *4*, 335–340.

Devaraja, T. N., Yusoff, F. M., & Shariff, M. (2002). Changes in bacterial populations and shrimp production in ponds treated with commercial microbial products. *Aquaculture*, *206*, 245–256.

Flegel, T. W. (2012). Historic emergence, impact and current status of shrimp pathogens in Asia. *Journal of Invertebrate Pathology*, *110*, 166–173.

Fuller, R. (1989). Probiotics in man and animals, a review. *Journal of Applied Microbiology*, *66*, 365–378.

Jayaram, K. C. (1981). *The freshwater fishes of India, Pakistan, Bangladesh, Burma, Sri Lanka: A handbook* (Vol. 475). Calcutta: Zoological Survey of India.

Jayasree, L., Janakiram, P., & Madhavi, R. (2006). Characterization of *Vibrio* spp. associated with diseased shrimp from culture ponds of Andhra Pradesh (India). *Journal of the World Aquaculture Society*, *37*, 523–532.

Karunasagar, I., & Ababouch, L. (2012). Shrimp viral diseases, import risk assessment and international trade. *Indian Journal of Virology*, *23*, 141–148.

Kathiresan, K. (2000). A review of studies on Pichavaram mangrove, Southeast India. *Hydrobiologia*, *430*, 185–205.

Kelch, W. J., & Lee, J. S. (1978). Antibiotic resistance pattern of Gram-negative bacteria isolated from environmental sources. *Applied and Environmental Microbiology*, *36*, 450–456.

Kirby, W. M., Bauer, A. W., Sheriss, J. C., & Turc, M. (1996). Antibiotic susceptibility testing by standardized single method. *American Journal of Clinical Pathology*, *45*, 493–496.

Kumar, K. M. V., & Kumara, V. (2011). Avifaunal diversity of mangrove ecosystem, Kundapura, Udupi district, Karnataka, India. *The Recent Research in Science and Technology*, *3*, 10.

Lazado, C. C., Caipang, C. M., & Estante, E. G. (2015). Prospects of host-associated microorganisms in fish and penaeids as probiotics with immunomodulatory functions. *Fish and Shellfish Immunology*, *45*, 2–12.

Manikandan, R., & Vijayakumar, R. (2016). Physico-chemical analysis of marine soils from different ecosystem palk strait coastal regions of Tamil Nadu, India. *Journal of Marine Science: Research & Development*, *6*(208), 1–5.

Manikandan, R., & Vijayakumar, R. (2015). Marine actinobacterial diversity from Palk Strait coastal region of Bay of Bengal. *Wide Spectrum*, *3*, 25–34.

Marmur, L. J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganism. *Journal of Molecular Biology*, *3*, 208–218.

Minotto, E., Luciana Pasqualini, M., Martha Trindade, O., & Teresinha, Sueli (2014). Enzyme characterization of endophytic actinobacteria isolated from tomato plants. *Journal of Advanced Scientific Research*, *5*(2), 16–23.

Mohanta, K. N., Mohanty, S. N., Jena, J. K., & Sahu, N. P. (2007). Protein requirement of silver barb, *Puntius gonionotus* finger lings. *Aquaculture Nutrition*, *13*, 1–10.

Moriarty, D. J. W. (1999a). Disease control in shrimp aquaculture with probiotic bacteria. In CR Bell (Ed.), *Microbial biosystems: New frontiers proceedings of the 8th international symposium on microbial ecology*Atlantic Canada Society for Microbial Ecology, Halifax, Canada.

Moriarty, D. J. (1999b). Control of luminous *Vibrio* species in penaeid aquaculture ponds. *Aquaculture*, *164*, 351–358.

Morinigo, M. A., Cornax, R., Castro, D., Jimenez-Notaro, M., Romero, P., & Borrego, J. J. (1990). Antibiotic resistance of *Salmonella* strains isolated from natural polluted waters. *Journal of Applied Microbiology*, *68*, 297–302.

Muraleedharan, H., Kannapiran, E., KalaiGandhi, V., & Michale, A. (2009). Antibiotic sensitivity test against *Vibrio* sp. isolated from Rameswaram Island. *Scientific Transactions in Environment and Technovation Journal*, *3*(2), 64–68.

Nates, F.S., (1994). Effect of brewer's yeast on growth and survival of white shrimp Litopenaeus vannamei.

Oskay, M., & Husniye, T. Y. (2015). Screening of yeast strains for pectinolytic activity effect of different carbon and nitrogen sources in submerged fermentations. *Journal of Biological Sciences*, *15*(3), 89–96.

Pandey, B., Ghimire, P., & Agrawal, V. P. (2004). *Studies on the antimicrobial activity of actinomycetes isolated from Khumbu region of Nepal*. Kathmandu, Nepal: Tribhuvan University. PhD dissertation.

Peters, R. I., & Long, G. E. (1973). Some alteration in haemolymph protein of *Hemiorapaus nudus* associated with temperature accumulation. *Comparative Biochemistry and Physiology*, *46*, 207–213.

Prabhu, V. A., Rajkumar, M., & Perumal, P. (2008). Seasonal variations in physico-chemical characteristics of Pichavaram mangroves, Southeast coast of India. *Journal of Environmental Biology*, *29*(6), 945–950.

Rengipipat, S., Rukpratanpom, S., Piyatiratitivorakul, S., & Menasaveta, P. (2000). Immunity enhancement in black tiger shrimp by a probiont bacterium (*Bacillus* sp. 11). *Aquaculture*, *191*, 271–288.

Sachidanandamurthy, K. L., & Yajurvedi, H. N. (2006). A study on physico–chemical parameters of an aquaculture body in Mysore City, Karnataka, India. *Journal of Environmental Biology*, *27*, 615–618.

Seibert, C. H., & Pinto, A. R. (2012). Challenges in shrimp aquaculture due to viral diseases: Distribution and biology of the five major Penaeid viruses and interventions to avoid viral incidence and dispersion. *Brazilian Journal of Microbiology*, *43*, 857–864.

Shen, W. Y., Fu, L. L., Li, W. F., & Zhu, Y. R. (2010). Effect of dietary supplementation with *Bacillus subtilis* on the growth, performance, immune response and antioxidant activities of the shrimp. *Aquaculture Research*, *41*, 1691–1698.

Singh, S., Pramod, K., Gopalan, N., Bhuvnesh, S., Kuhad, R. C., & Hotam Singh, C. (2012). Isolation and partial characterization of actinomycetes with antimicrobial activity against multidrug resistant bacteria. *Asian Pacific Journal of Tropical Biomedicine*, *2*(2), 1147–1150.

Srivibool, R. (2000). Antimicrobial activity of *Actinomadura* isolates from tropical island soils. *Actinomycetes*, *10*, 10–12.

Stentiford, G. D., Neil, D. M., Peeler, E. J., Shields, J. D., Small, H. J., & Flegel, T. W. (2012). Disease will limit future food supply from global crustacean fishery and aquaculture sectors. *Journal of Invertebrate Pathology*, *110*, 141–147.

Sun, C. H., Li, F. N., & Cai, Z. (2017). Brief introduction of mangrove researches including drug discovery granted by National Natural Science Foundation of China from 1986 to 2016. *Chinese Journal of Antibiotics*, *42*(4), 241–248.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, *28*, 2731–2739.

Tanner, C. C., Craggs, R. J., Sukias, J. P., & Park, J. B. (2005). Comparison of maturation ponds and constructed wetlands as the find stage of an advanced pond system. *Water Science and Technology*, *51*, 307–314.

Tanvir, R., Sajid, I., Hasnain, S., Kulik, A., & Grond, S. (2016). Rare actinomycetes *Nocardia caishijiensis* and *Pseudonocardia carboxydivorans* as endophytes, their bioactivity and metabolites evaluation. *Microbiological Research*, *185*, 22–35.

Usha Kiranmai, G., Raja Sekhar, P. S., & Hema Latha, S. (2015). Analysis of physico-chemical parameters of mangroves and wetland soils of Visakhapatnam coast, Bay of Bengal, India. *International Journal of Innovative Research and Creative Technology*, *2*(2), 60–64.

Vaijayanthi, G., & Vijayakumar, R. (2014). Physico-chemical analysis of marine sediment soils from Muthupet mangrove environments in Tamil Nadu, India. *International journal of Advanced Life Sciences*, *7*, 417–423.

Veeramani, S., Babu, S., Tharanya, M., Shijila Rani, A. S, Ambikapathy, V., & Panneerselvam, A. (2019). Screening of actinobacteria by enzyme production and antimicrobial properties from marine environment. *Journal of Emerging Technologies and Innovative Research*, *6*(6), 340–348.

Vilas, M. A., & Ghosal, A. (2015). Water and soil quality analysis of selected areas of Sunderban and mapping using GIS technique. *International Journal of Research in Chemistry and Environment*, *5*(4), 44–59.

Wang, Y. B., & Xu, Z. R. (2004). Probiotics treatment as a method of biocontrol in aquaculture. *Feed Research*, *12*, 42–45.

Yanbo, W., & Qing, G. U. (2015). Effect of probiotics on white shrimp (*Penaeus vannamei*) growth performance and immune response. *Marine Biology Research*, *6*, 327–332.

Yuan, L., Zhang, Y., Yu, L., Sun, C., Wei, Y., Liu, H., et al. (2010). *Actinopolymorpha cephalotaxi* sp. nov., a novel actinomycete isolated from rhizosphere soil of the plant *Cephalotaxus fortunei*. *International Journal of Systematic and Evolutionary Microbiology*, *60*, 51–54.

Chapter 22

Probiotics as a Growth Promotant for Livestock and Poultry Production

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1 Introduction

The poultry industry is a highly dynamic and evolving sector in response to rapidly increasing for poultry products including high demand for meat and eggs mainly driven by boosting human population growth and urbanization. The chickens have the potential to furnish income and food to rural poor by converting accessible, nonconventional feed resources found around households into exploitable protein in the form of eggs and meat (Atela, Mlambo, & Mnisi, 2019). The foremost requirement of human consumption for protein is directly met by the poultry industry in the form of chicken meat. As of 2016, the overall global production of poultry eggs and the meat was approximately 74 and 119 million tons, respectively, and the chicken meat production was predicted to increase to 122 million tons by November 2018. Although it has been estimated that by 2050, the global human populace is envisaged to reach approximately 10 billion (Sood et al., 2019). Providing ample and nutritious food for such a huge population highlights the significance of the world's agriculture system. Indeed, the Food and Agriculture Organization (FAO) of the United Nations projects that food production will have to increase by 70% over the same time frame (FAO/WHO, 2009). Note that as with any presumption, there are several basic hypotheses and uncertainties linked with the reported number, though it is assured that they will be necessitated for worldwide food production to augment substantially in the foreseeable future.

The anticipated rise in income globally will increase demands for not only more food but also for the better quality of food, leading to increase intake of animal protein (FAO/WHO, 2009). The requirement of more high-quality protein foods will have to be met by increases resulting from animal production systems. Therefore, sustainable poultry productions are needed to be intensified to the increasing human population.

2 Exploitation of antibiotics in poultry production

During the intensification of the chicken production process, the advent of antibiotics and their utilization for the treatment of clinical manifestations of infections had a substantial role in the advancement and prosperity of the poultry industry. Additionally, to direct application in the control of disease, subtherapeutic doses of antibiotics given in poultry feed have been revealed to associate with the eminent health status of the poultry and enhanced growth performance employing dynamic weight gain and improved meat production. Therefore, the antibiotics have been successfully employed in poultry production as "growth promotants" (Harms, Ruiz, & Miles, 1986; Engberg, Hedemann, Leser, & Jensen, 2000; Emami, Samie, Rahmani, & Ruiz-Feria, 2012; Chattopadhyay, 2014; Mehdi et al., 2018) and also defend the health by altering the immune status of broiler chickens (Lee et al., 2012). However, the mechanism remains unclear, but possibly the antibiotics are acted by remodeling the diversity of microbes and their relative abundance in the Gastro-Intestinal (GI) tract to confer an optimal growth of microbes. Besides, the reduction of the beneficial microbial population after the usage of antibiotics has been described in several studies (Zhou et al., 2007; Danzeisen, Kim, Isaacson, Tu, & Johnson, 2011; Lee et al., 2012). It has been proposed that the normal flora of the GI tract was able to produce the bile salt hydrolase (BSH) enzyme. The diminishing of the beneficial population in antibiotic-fed poultry probably

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00022-8
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decreases of BSH enzyme activity in the GI tract, that would enhance the relative abundance of conjugated bile salts, which modifies host lipid metabolism leading to decreased digestibility of fat and thereby increases the poultry weight gain (Lin, Hunkapiller, Layton, Chang, & Robbins, 2013). A benefit is that it improves the rate and efficiency in the gain of body weight, reduction in mortality and morbidity, controlling the occurrence of subclinical diseases as well as modifying the microbiota of the intestine (Torok, Allison, Percy, Ophel-Keller, & Hughes, 2011). Besides, the antibiotics might minimize the microbial load in the GI tract leading to more availability of nutrients to the host (Brisbin, Gong, & Sharif, 2008). It was also anticipated that the net effect of antibiotics in feed in the poultry industry was a 3%–5% rise in growth as well as feed conversion efficiency (Dahiya, Wilkie, Van Kessel, & Drew, 2006; Gadde, Kim, Oh, & Lillehoj, 2017). Subsequently, the exploitation of the inclusion of antibiotics to the poultry feed to stimulate growth has turned into global practice.

2.1 Antibiotics and microbial resistance

As a consequence, it can be noted that growth-promoting antibiotics take part in a decisive role in contributing to the economic efficiency of livestock production (Wierup, 2000). Despite the well-established positive effects of antibiotics for augmenting the growth rate, decreasing mortality, and developing resistance to disease, their use was also known to be linked with several drawbacks and challenges. Concerns subsist that the use of growth-promoting antibiotics in poultry is cited as a prime factor that resulting in promoting the emergence of antimicrobial-resistant bacteria, posing an imminent threat to human health. Data in the literature also generally evidence that the use of antibiotic growth promoters in livestock production leads to microbial resistance in treated animals (O'Brien, 2002). Antibiotic resistance is defined as the ability of microbes to multiply in the existence of an antibiotic that usually inhibits or kills microorganisms of the same species (Mehdi et al., 2018). Such resistance is occurring by decreasing outer membrane permeability by down regulation of porins to reduced antibiotic uptake, methylation of the target to prevent drug binding, genetic modification of target mutation or acquisition of genes transmitted by mobile genetic elements such as transposons, integrons, plasmids, or phages and evading the pathways which affect the antibiotic or overexpression of efflux pumps for active transport of antibiotics out of the cell (Atterbury et al., 2011).

2.2 Impact of antibiotic on the environment and consumer health

In addition, antibiotic-related contaminants such as antibiotic-resistant microbes, antibiotic resistance genes, and antibiotic drugs are classified as emerging environmental pollutants (Keen & Patrick, 2013). Soil and water resources are considered as important reservoirs for acquisition and extent of antibiotic resistance genes by bacteria. Inclusion of antibiotics in animal feed or drinking water is not entirely metabolized in the GI tract of poultry and about 70% is the antibiotics is excreted in its unmetabolized form (Kümmerer, 2009). Residues of antibiotics in poultry wastes, when used as manure, can introduce antibiotics, resistant strains, and antibiotic resistance genes in the soil environment (Furtula et al., 2010; Franklin et al., 2016). In the soil ecosystem, antibiotics act differently based on the physicochemical characteristics, soil properties, and climatic conditions. The accumulation of antibiotics in the soil can lead to a significant alteration in the soil structure and activity of the rhizosphere soil microbiota (Yang, Zhang, Zhu, & Zhang, 2009). Moreover, several investigators have also documented that the accumulation of drug residues in plants for which poultry litter was exploited as manure (Kumar, Gupta, Baidoo, Chander, & Rosen, 2005; Tasho & Cho, 2016). The antibiotics also influence the microbial community residing in the soil environment which in turn can affect the degradation of organic matter. Antibiotic residuals in soil promote the fastidious proliferation of resistant microbes and enhance the diversity of antibiotic resistance genes that can be subsequently transferred vertically or horizontally (Ding & He, 2010; Jechalke, Heuer, Siemens, Amelung, & Smalla, 2014). From contaminated soils, these pollutants can also penetrate ground and surface water resources by leaching or water run-off via livestock wastewater and pose a high risk for groundwater and the microbial composition (Caracciolo, Topp, & Grenni, 2015). Furthermore, the presence of antibiotics residues in the water contributes to the maintenance, emergence, and distribution of microbial populations with a modest level of resistance and ready to evolve toward resistance. In the aquatic ecosystem, the antibiotics might completely or partially degraded or it may be accumulated in tissues of the aquatic organisms (Gaw, Thomas, & Hutchinson, 2014), that could subsequently be consumed by humans. The spreading of antibiotic resistance in the environment (from "farm-to-fork") has been portrayed in Fig. 22.1. Nevertheless, by-products of antibiotics in the environment remain active and are capable of potentially more toxic, stable, and mobile rather than their parent compounds. Besides bioresistance, the exploitation of antibiotics has led to the retention of drug residues in poultry products which causes adverse impacts on human health.

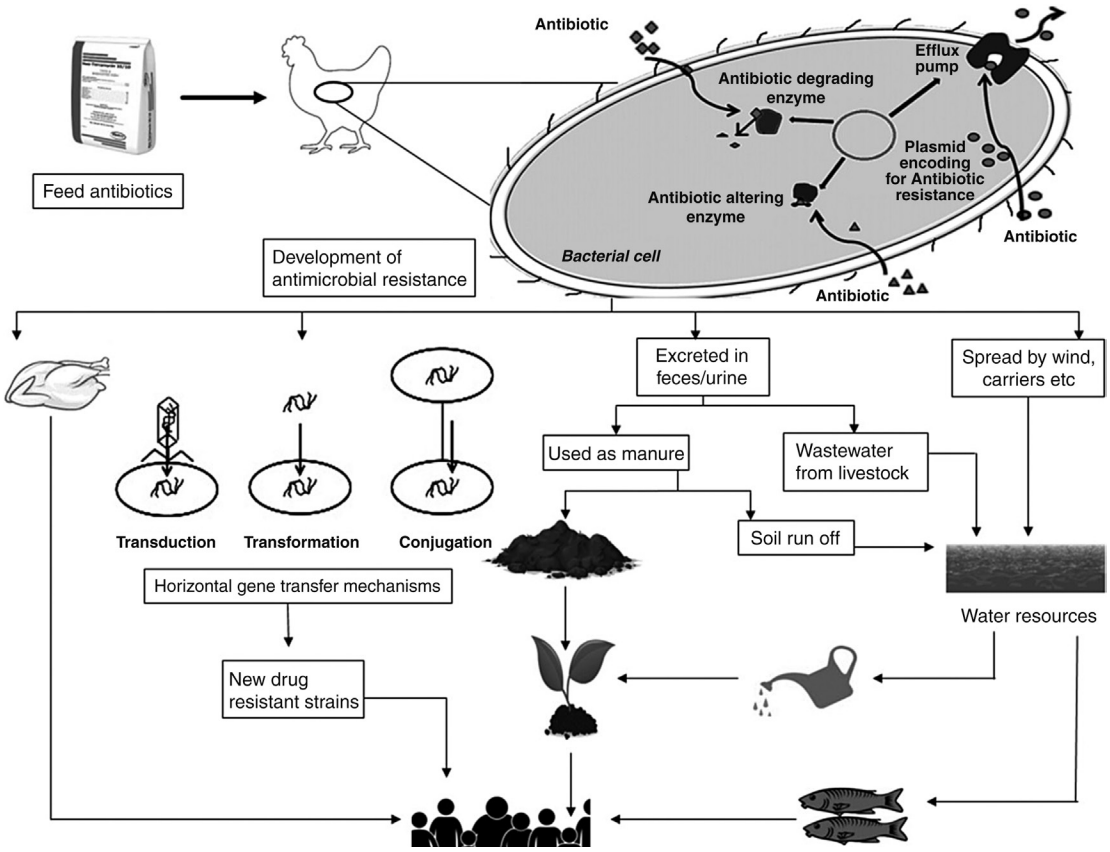


FIGURE 22.1 Dissemination of antibiotic resistance in environment (Suresh et al., 2018).

2.3 Alternatives to the usage of antibiotics

Beyond the positive attributes, the threat pertaining to the development of bacterial resistant and transference of antibiotic resistance genes from poultry to human microbiome led the European Union to forbid the appliance of antibiotics as growth promotants since January 2006, which was followed by the other parts of the world (Yegani & Korver, 2008). On the other hand, the exclusion of antibiotic growth promoters from poultry diet has resulted in health performance problems and an increase in the occurrence of poultry diseases such as subclinical necrotic enteritis and dysbacteriosis (Huyghebaert, Ducatelle, & Van Immerseel, 2011). Owing to the fact, the growing concerns over the antibiotics growth promoters use, the quest for novel alternate to mitigate antibiotic use in poultry production has grown over the years. To date, the need to search alternatives for antibiotic growth promoter is therefore imperative. As a consequence, the great deal of research has intended on the development of alternatives to antibiotics sustain or augment chicken health and performance.

Due to the consumers' demands and worries toward the detrimental effects of antibiotic growth promoter, the quest for novel replacements to mitigate antibiotic use in animal agriculture has grown over the years. In the past two decades, a great deal of research has focused on the development of antibiotic alternatives to maintain or improve poultry health and performance. At the same time, an ideal alternative must have similar positive effects of antibiotics that assure optimal poultry performance and enhance nutrient availability (Huyghebaert et al., 2011). In view of the proposed mechanism the action of growth-promoting antibiotics (altering the microbiome and immunomodulating activities), a realistic alternative must acquire both of these properties in addition to having a beneficial impact on feed conversion and growth (Huyghebaert et al., 2011; Seal, Lillehoj, Donovan, & Gay, 2013). These alternatives aim to sustain a no mortality rate, a high-quality level of poultry yield while protecting the environment and consumer health. A great extent of research has been performed to look for green additives with analogous positive effects of antibiotic growth promoters. Consequently, the poultry industry must focus on an alternative to antibiotics for retaining the health and enhancing growth performances under profitable conditions are urgently needed. Growing interest has focused on the "exploitation of live microbes as probiotics" is recommended as a valid alternative to the prophylactic and growth-promoting application of antibiotics. They unveiled the attributes of improving poultry productive performance, augment the intestinal microflora of poultry by replenishing

suppressed pathogenic bacteria colonization and retaining the balance of microbiota of the digestive tract of the host. Besides, they are natural, harmless microbes and do not leave behind any hazardous antibiotic residue in the edible portions of food-producing animals after being fed to them (Latha, Vinothini, Calvin, & Dhanasekaran, 2016).

3 Probiotics: definition, concepts, and history

Probiotics are live microorganisms that are believed to provide health benefits to the host. The word "probiotics" originated from the Greek words "pro" and "bios" which means "for life", that is, opposed to "antibiotics" which means "against life." They are also called as functional food, friendly bacteria, good bacteria, protective bacteria, and effective microbiota. The term "probiotics" was first introduced in 1953 by Kollath to depict "organic and inorganic food supplements applied to restore health to patients suffering from malnutrition" and the probiotic concept was first proposed by Lilly and Stillwell (1965) to imply the "substances secreted by one microorganism, which stimulated the growth of another." A few years later, the definition was improved in the perspective of animal feeds as "organisms and substances which contribute to intestinal microbial balance" (Parker, 1974). Fuller (1989) revised the probiotics definition as "live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance," which emphasizes the necessity of viability of probiotics and establishes the aspect of beneficial effects on the host. This definition has been widely accepted and used by the entire scientific world. Afterwards, certain modifications were proposed by several researchers (Salminen, 1996; Verschuere, Rombaut, Sorgeloos, & Verstraete, 2000) to trim the original definition of probiotics. The new definition put forward by Food and Agriculture Organization (FAO) and the World Health Organization (WHO) jointly stated probiotics as "live microorganisms, providing health benefits for the host, when they are administered in adequate amounts" (FAO/WHO, 2001). This definition is widely accepted and approved by the International Scientific Association for Probiotics and Prebiotics.

The probiotics history was begun with the invention of microbes and the consumption of fermented foods for nutritional and therapeutic uses. Tissier (1906) was the first to report on the isolation of dominant probiotic *Bifidobacterium* species (later named as *Bacillus bifidus communis*) from feces of breast-fed babies, and he observed clinical advantages of treating diarrhea in infants with bifidobacteria. Metchnikoff (1907) documented that fermented milk products containing lactic acid bacteria, that is, "Bulgarian bacillus" afterward called *Lactobacillus delbrueckii* subsp. *bulgaricus* are mainly responsible for the long life of Bulgarians. The first non-Lactic acid bacteria (LAB) probiont was outlined in 1917 by a German professor Alfred Nissle, who isolated a nonpathogenic *Escherichia coli* strain from the feces of a First World War soldier, who did not develop enterocolitis during a severe outbreak of shigellosis. He also used the *E. coli* Nissle 1917 strain in acute cases of infectious intestinal diseases with considerable success (Nissle, 1959). Probiotic treatment accomplished the main footstep in the direction of reality in 1930 when a Japanese microbiologist Shirota first discovered the survival of *Lactobacillus casei* strain Shirota in the passage of the GI tract after its ingestion (Shirota, Aso, & Iwabuchi, 1966). In addition, clinical trials testing the efficiency and protection of probiotic products and identification of uncultivable microbes using metagenomic analysis are the major crucial points of present probiotic research, which are evident from the increased number of publications on probiotics related to human and animal health enhancement.

3.1 Microbes used as animal probiotics

Probiotics are defined as the microorganisms that exhibit diverse beneficial effects on the ingested host health and comprise LAB, *Bifidobacterium*, *Bacillus*, and yeast. Among those, *Lactobacillus*, under the LAB group, was the most primitive discovered probiotic. This genus of rod-like-shaped Gram-positive bacteria encompasses 183 recognized species, exploited in numerous industrial processes (de Melo Pereira, de Oliveira Coelho, Júnior, Thomaz-Soccol, & Soccol, 2018). The genera *Lactobacillus*, including *L. acidophilus*, *L. fermentum*, *L. plantarum*, *L. casei*, *L. paracasei*, *L. reuteri*, and *L. rhamnosus*, is the predominant LAB group in animal gastrointestinal and digestive systems, acquiring verified action in the maintenance and recovery of animal health.

Other LAB genera with confirmed probiotic effects consist of *Streptococcus*, *Lactococcus*, *Enterococcus*, *Pediococcus*, *Weissella*, and *Leuconostoc.* Subsequently, the genus *Bifidobacterium* comprises 30 species, where 10 are from humans, 17 from animals, 2 from residual waters, and one from fermented milk (Picard et al., 2005; Russell, Ross, Fitzgerald, & Stanton, 2011). Owing to the high DNA content of guanine and cytosine (ranges from 42% to 67%), the genus of *Bifidobacterium* was inserted into the order of Actinomycetales. The diverse probiotic benefits have been reported for *B. adolescentis*, *B. animalis*, *B. infantis*, *B. pseudolongum*, *B. bifidum*, *B. breve*, *B. thermophilum*, and *B. longum.* The genus *Bacillus* is also used as a probiotic in the food and pharmaceutical industry (Ripert et al., 2016; Liu, Wu, Chu, & Wu, 2018). The major facet of this Gram-positive, aerobic bacteria group is the formation of endospores (Cutting, 2011) that retain the viability for long shelf life.

On the other hand, a few species such as *Bacillus anthracis*, *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus pseudomycoides*, and *Bacillus weihenstephanensis* were identified to synthesize enterotoxins representing a safety concern. The yeasts consist of a large and heterogeneous group of eukaryotic microorganisms that are ubiquitous in natural environments, including the GI tract of humans, plants, animals, airborne particles, and food products (Foligné, Dewulf, Vandekerckove, Pignède, & Pot, 2010). The elevated content of proteins, vitamin B, traces minerals, and various immune-stimulants (proteases, β-glucans, and mannan oligosaccharides) has augmented interest in the utilization of yeasts as a probiotic (Garcia Hernandez et al., 2012; Gil-Rodríguez, Carrascosa, & Requena, 2015; Fadda, Mossa, Deplano, Pisano, & Cosentino, 2017; Arévalo-Villena, Fernandez-Pacheco, Castillo, Bevilacqua, & Pérez, 2018). In addition, yeasts also have the advantages of nonsusceptibility to antibiotics and excellent tolerance for industrial processing conditions (Abdel-Rahman, Tashiro, & Sonomoto, 2013; Fleet, 2006). According to the European Food Safety Authority (EFSA), *Saccharomyces boulardii* has a Qualified Presumption of Safety (QPS) status and is the most widely used probiotic (Hudson et al., 2016). Nevertheless, some other potential probiotic yeast strains such as *Kluyveromyces*, *Pichia*, *Rhodotorula*, *Schizosaccharomyces*, and *Candida* were constantly being identified (Tokuhiro, Ishida, Kondo, & Takahashi, 2008; Lane & Morrissey, 2010; Banjara, Suhr, & Hallen-Adams, 2015). The products of probiotics might contain single or more selected microbial strains. The microorganisms intended as feed supplements in the EU are generally bacteria. The most frequently studied microbes for probiotic usage in poultry belong to the genera *Lactobacillus*, *Enterococcus*, *Bifidobacterium*, *Bacillus*, *Pediococcus*, *Enterococcus*, *Propionibacterium*, *Streptococcus*, and *Leuconostoc*. In addition, some fungi (*Aspergillus*) and yeast strains (*Saccharomyces cerevisiae* and *S. boulardii)* were exploited as probionts. A number of probiotic microorganisms intended for poultry and other livestock production and very few of them are indexed in Table 22.1.

Administration of probiotic feed enhances growth, feed efficiency, and intestinal health of poultry. This enhancement is attained by dropping intestinal pH and improving gut bacteria composition and digestive activity. Mechanisms of action of probiotics comprise the diminution of metabolic reactions that produce toxic substances, stimulation of endogenous enzymes, and synthesis of vitamins (Hassanein & Soliman, 2010). Probiotics have also been known to antimicrobial effect on pathogenic bacteria in the gut, by producing antimicrobial substances called bacteriocin and employing competitive exclusion (CE) to prevent pathogen colonization. Probiotics exert to modify the intestinal epithelial architecture by increasing the production of mucus and preventing the adherence and consequent colonization of the gut epithelium by pathogenic bacteria (Smirnov, Perez, Amit-Romach, Sklan, & Uni, 2005; Hardy, Harris, Lyon, Beal, & Foey, 2013). Furthermore, probiotics can also retain the integrity of epithelial tight junctions by upregulating genes that code for junction proteins that are responsible for tight junction signaling and restoration of mucosal integrity (Syngai et al., 2016).

TABLE 22.1 Probiotic microorganisms mostly intended for poultry and other livestock.

Type: *Lactobacillus*	Type: *Bifidobacterium*	Other lactic acid bacteria	Other microorganisms
L. brevis	*B. animalis*	*Enterococcus faecalis*	*Bacillus cereus*
L. casei	*B. longum*	*Enterococcus faecium*	*Bacillus licheniformis*
L. crispatus	*B. pseudolongum*	*Lactococcus lactis*	*Bacillus subtilis*
L. farciminis	*B. thermophilum*	*Leuconostoc citreum*	*Propionibacterium freudenreichii*
L. fermentum	*B. bifidum*	*Leuconostoc lactis*	*Saccharomyces cerevisiae*
L. murinus	*B. breve*	*Leuconostoc mesenteroides*	*Saccharomyces boulardii*
L. gallinarum	*B. adolescentis*	*Pediococcus acidilactici*	*Saccharomyces pastorianus*
L. paracasei	*B. infantis*	*Pediococcus pentosaceus*	*Kluyveromyces fragilis*
L. pentosus		*Streptococcus infantarius*	*Kluyveromyces marxianus*
L. plantarum		*Streptococcus salivarius*	*Aspergillus oryzae*
L. reuteri		*Streptococcus thermophilus*	*Aspergillus niger*
L. rhamnosus		*Sporolactobacillus inulinus*	*Candida famata*
L. salivarius		*Weissella cibaria*	*Streptomyces* sp.
L. acidophilus		*Weissella confuse*	*Rhodococcus* sp.
L. gasseri			
L. helveticus			

A number of mechanisms have been reported for toxin inhibition by probiotic species, such as the production of a protease to degrade the toxin and its receptor, reduction of cyclic adenosine monophosphate (cAMP) production that eventually prevents diarrhea and binding of the toxin to receptors on the probiotic surface (Khan & Naz, 2013). Various scientific teams have revealed an extensive range of studies and analyses regarding the benefaction of probiotic administration in the development of the broiler growth rates. The probiotic microbes also have positive effects on poultry meat quality by improving the protein and fat contents of the meat (Hassanein & Soliman, 2010; Popova, 2017). They mainly enhance pH, color, fatty acid profile, chemical composition, water retention capacity, and oxidation stability (Popova, 2017). Recognition of these astounding beneficial effects of probiotics has led to mounting interest in using these microbes for poultry husbandry.

4 Probiotics for poultry nutrition: a glance on the market

Poultries are exposed to the environment-related stress that encompasses diet, rearing methods, weaning, and various issues. Various factors might cause dysbiosis in the intestinal environment reflecting a high risk for infections. Probiotics and their use in poultry nutrition and direct feeding related to their confirmed effectiveness by retaining the equilibrium of the intestinal homeostasis. The ingestion of probiotic strains could have a vital impact on the absorption and utilization of feed, on metabolism, and numerous parameters like daily and total body weight gain of poultry. The ready-made probiotic inclusions for poultry nutrition may be composed of single or several strains of bacterial species (Zommiti, Chikindas, & Ferchichi, 2019). In such formulas, their efficacy is directly connected to two main key factors: the precise selection of the strain(s) and the unitary dose containing the proper count of viable cells.

To conserve the attributes of probiotic preparations and formulas, these beneficial microorganisms ought to be stored and used according to the manufacturer's instructions. Owing to the content of viable microbes, the probiotic preparations are liable to unfavorable conditions, such as temperature and light. No other substances must be used while probiotics are administered like water used for dilution contains no chlorine or other disinfectants. Additionally, there are different forms of probiotics like liquid, powder, gel, paste, and granules, which are usually available in the form of capsules, tablets, sachets, etc. (Iannitti & Palmieri, 2010). The utilization of hydroxypropyl methylcellulose phthalate 55 as a matrix for tablet form provided better viability of probiotic in poultry (Jiang et al., 2017). The dry dosage form of probiotics possesses longer shelf life on storage as well as exhibited excellent tolerability to the intestinal environment. A range of probiotic products is available in the market, which are summarized in Table 22.2

TABLE 22.2 Commercial probiotics used in poultry (Markowiak & Śliżewska, 2018).

Trade name	Microorganisms	Manufacturer
Acid-Pak-4-Way®	*Lactobacillus acidophilus, Enterococcus faecium*	Alltech
Avian PAC	*Streptococcus faecium, L. acidophilus*	Soluble Loveland Industries
Biogen D	*Bifidobacterium bifidum, L. acidophilus, Pediococcus faecium*	Bio-Gen
Doctor Em®	*Lactobacillus paracasei, Lactobacillus plantarum;, Lactococcus lactis, Saccharomyces cerevisiae*	Biotron
Ecobiol	*Bacillus amyloliquefaciens*	Norel Animal Nutrition
Enviva™ Pro	*Bacillus subtilis*	DANISCO Animal Nutrition
FloraMax-B11	*Lactobacillus salivarius, Pediococcus parvulus*	Pacific Vet Group
GalliPro®	*B. subtilis*	Evonik Industries
Galvit Probiotyk	*E. faecium*	Galvit
Microguard	*B. licheniformis, B. megaterium, B. mesentricus, B. polymyxa, B. subtilis, Saccharomyces boulardii; B. bifidum; L. acidophilus, L. bulgaricus, L. plantarum; S. faecium*	PeterLab Holdings
PrimaLac	*Bifidobacterium bifidium, Bifidobacterium thermophilus, E. faecium; L. acidophilus, L. casei*	Star Labs, Inc.
Probion	*B. subtilis, Clostridium butyricum, L. acidophilus*	Woogene B&G Co. Ltd.

4.1 Selection of probiotics

To date, heaps of microbes are used as a probiotic by selecting the instinctive intestinal microbiota of farm animals. Nevertheless, the determination of an optimal species for probiotic practice is entirely empirical, and several criteria must be considered for the selection of appropriate probionts (Saarela, Mogensen, Fonden, Mättö, & Mattila-Sandholm, 2000).

In most cases, the huge number of isolated microbes leads to the need to use a "step-by-step approach," consisting of a series of tests to gradually reduce the number of probiotic candidates. At the end of this method, the microbes that present the highest number of functional properties, and, concomitantly, without any negative traits, are selected. The selection criterion must be sensibly based on the safety of the strain (nonpathogenic), functionality (survival under gastric and intestinal conditions, colonization and proliferation in the intestinal epithelium, production of bioactive substances, immune stimulation, and removal of toxic compounds), and technological suitability (stability and viability in processes).

4.1.1 Origin of the probiotic strain

Host specificity is recommended as the foremost criterion for selection of probionts since it is one of the most desirable properties for probiotic strains (Saarela et al., 2000). Hence, the probiotic microbes should be isolated from the same species as their intended host, which means that they should preferentially originate from the target animals for host-specific responses. Such probionts are claimed to have the preeminent chance to survive and colonize the GI tract allowing the beneficial microbiota to thrive, thus making their use ethically more acceptable and potentially more efficient than other exogenous strains.

4.1.2 Stress tolerance ability

Initially, the candidate strains are assessed to cope with the stress conditions of the stomach (low pH and pepsin) and intestines (pancreatin and bile salts), as well as mild heat-shock caused by the host body temperature, so that they cannot be easily killed by the defense mechanisms of the host during their transit through the GI tract.

4.1.3 Colonization ability

The next step is to evaluate whether the potential probiotic candidates can colonize the GI tract epithelial cells. The probiotic bacteria encounter mucus and epithelial cells in the small intestine from where they can be easily eliminated by the process of peristalsis. Consequently, to deliver the beneficial effects over a prolonged period, probiotics must colonize the GI tract. In general, autoaggregation and cell surface hydrophobicity are used as measures of bacterial adhesion because they are directly related to their adhesion ability to the intestinal epithelium (Del Re, Sgorbati, Miglioli, & Palenzona, 2000).

4.1.4 Safety assessment

The possibility of infection when administering live microbes to the feed must be evaluated, and the conclusion that "probiotics are safe" should not be fully considered (Culligan, Hill, & Sleator, 2009). Some initiatives by the European community (The European Union Novel Food regulation, QPS, and PROSAFE), United States (FDA and WHO), and Canada (Health Canada: NHPR) have been decided to establishing criteria for the safety assessment of probiotics. General recommendations include records of isolation history, taxonomic identification, and absence of virulence, infectivity, toxicity, and transferable antibiotic resistance genes (Sanders et al., 2010). Antibiotic resistance of probiotics is measured as a safety issue when the risk of gene transfers is present (Gueimonde, Sánchez, de los Reyes-Gavilán, & Margolles, 2013). If the resistance is intrinsic (i.e., acquired) as a result of a chromosomal mutation, probiotic bacteria do not constitute a safety concern themselves since the antibiotic resistance is only passed onto the next generation via the organisms genetic material; in this case, the resistant probiotic strains can be used concurrently or following antibiotic therapy to restore the gut microbiota (Galopin, Cattoir, & Leclercq, 2009; Hammad & Shimamoto, 2010; Gueimonde et al., 2013). Horizontal gene transfers by mobile elements such as plasmids, conjugative transposons, integrons, and bacteriophages likely to be carried out with probiotics that leads to the formation of antibiotic resistant strains. Therefore, a critical feature in studying antibiotic resistance in probiotic bacteria is to separate intrinsic resistance from acquired resistance (Sanders et al., 2010). Although, in common, there is no risk of the transfer of these genes to host gut commensals since the genes are not plasmid-encoded, it is a phenomenon that does not represent a safety concern (Zhang et al., 2009; Senan, Prajapati, & Joshi, 2015). Moreover, the probiotic strains must not have any pathogenic characters including the production of hemolysis, gelatinase, or DNAse.

4.1.5 Antipathogenic activity

Antagonistic activities of probiotic strains are indispensable to eradicate or reduce infection with pathogenic microbes. The synthesis of antimicrobial substances is often linked with antagonistic activities, and this is the prime functional trait considered when selecting prospective probiotics. Most of the probiotic strains accomplish this property by producing several antimicrobial substances, including lipopeptides, surfactin, bacteriocins, and bacteriocin-like inhibitory substances. These antimicrobial agents are typically active against Gram-positive pathogens, but few demonstrate activity against Gram-negative pathogens as well as fungal pathogens (Kerr, 1999; Teo & Tan, 2005; Khochamit, Siripornadulsil, Sukon, & Siripornadulsil, 2015). The antagonistic behavior of probiotics also includes the production of organic acids that decreasing pH and CE of pathogens for nutrients and adhesion sites.

4.1.6 Host-associated functional effects

In addition to these basic probiotic characteristics, microbes must have established several functional properties that could promote animal health and welfare. Thus, the probiotic microbes should produce a variety of extracellular enzymes including amylase, protease, lipase, phytase, cellulase, and xylanase. These enzymes have been used in animal nutrition to assist in the digestion of feed and improve nutrient absorption. The probiotic candidate must exhibit BSH activity, cholesterol-reducing ability, and antioxidant activity that may exert beneficial health effects by lowering the serum cholesterol levels and relieving oxidative stress.

4.1.7 Synthesis of functional molecules

Probiotics have cell components and secrete functional molecules, including short-chain fatty acids, peptides, exopolysaccharides, essential vitamins, and minerals, which confer a health benefit on the host.

4.1.8 Industrial requirements and technological attributes

Probiotic inclusions are considered to be medical or pharmaceutical products when aspects related to health, such as the diagnosis of a disease, therapy, prevention, or relief, are claimed. Thus far, EFSA has been abandoned all health claims made for probiotics, categorizing them as food supplements or dietary supplements (Sanders, Shane, & Merenstein, 2016). In this sense, the chosen probiotic must not have adverse effects on the taste or aroma of the product or enhance the acidity over the shelf life (Champagne, Gardner, & Roy, 2005; Ranadheera, Evans, Adams, & Baines, 2012; Goodarzi, 2016). Furthermore, the probiotic ought to survive food-processing stressors, like fermentation, harvesting, freeze-drying, and variations in temperature, pH, and oxidative and osmotic stress during storage. Lastly, from a safety outlook, probiotic strains must be genetically stable to avoid emerging pathogenicity or the loss of productivity. For probiotic efficacy, the viability of 10^6–10^9 CFU g^{-1} by the time of consumption is required. Probiotic benefits can be demonstrated using in vitro and in vivo methods. Although many probiotic effects have been shown using in vitro methods, these must be validated in vivo. Besides, with the increasing number of researches in the field of probiotics, their beneficial efficacies on poultry are explored one after another. Thus far, numerous microbes are fulfilling the above-stated selection criteria and are exploited as probiotics in chicken production. The results were ample and promising, though to confront the continuous emergence of various enteric diseases and to control the rapid dissemination of Multi drug resistant (MDR) pathogens and to promote the better growth performance of the target host, the search for a novel probionts is crucial.

5 Role of probiotics in poultry

Poultry have coevolved with microbial components that outnumber their own body cells and are known to provide genes that contribute significant functions in the colonized organs (De Jesus-Laboy et al., 2011). Primarily, the microbiota of GI tract comprising resident and transient floras play a crucial role in metabolic, nutritional, physiological, and immunological processes related to the well-being of animals. Among many important functions, the gut microbiota can convert feedstuffs into microbial biomass, and the fermentation end products can be utilized by the host animal (Kong, Teather, & Forster, 2010). A huge number of microbial types are prevailing in animal gut; however, most of them are uncultivable, and also the relationship between the gut microbiota and host is remaining unclaimed due to the complexity of internal ecological systems. Moreover, the number and dominance of microbial species that persist and colonize the GI tract usually fluctuate between individuals and are determined by a combination of host-specific and environmental factors such as age, gender, diet, and genotype.

Generally, probiotic microorganisms have optimistic effects on poultry performance, and it was proved from the reports of so many researchers (Peric et al., 2010; Kral, Angelovicova, Mrazova, Tkacova, & Kliment, 2011; Nunes et al., 2012). They stated that improvement in broilers performance with probiotics supplementation could be acquired by protection against pathogens, increased digestibility and nutrients absorption, synthesis of vitamins and short chain fatty acids, and improved mucosal immunity (Fig. 22.2). They are normally achieved by activation of bacterial digestive enzymes, maintenance of beneficial microbial population, and improvement in healthy environment of GI tract via nourishing the enterocytes, improving ileal mucosal development, and reinforcing mucosal barrier function through maintaining epithelial integrity (Taklimi, Lotfollahian, Shahne, Mirzaei, & Alinejad, 2012; Seifi, Shirzad, & Habibi, 2013). Numerous studies have revealed that different probiotics promote the growth performance of broilers. For instance, the final body weight and daily weight gain were enhanced by a strain of *Bacillus coagulans* ZJU0616 (Zhou, Wang, Gu, & Li, 2010) and by the inclusion of a probiotic *Clostridium butyricum* (Zhang et al., 2016). Parallel conclusions were also drawn when a probiotic cocktail such as *L. acidophilus*, *Bacillus subtilis*, *S. cerevisiae*, and *Enterococcus faecium* were supplemented to broilers. Thus, the positive effects could be linked with specific species of probiotics.

The accurate mechanism underlying the growth-promoting efficacy of probiotic is vague, but it is perceptible that probiotic functions by altering the GI tract microflora and maintaining the dynamic equilibrium of the microbiota (Mookiah, Sieo, Ramasamy, Abdullah, & Ho, 2014). These beneficial effects might decrease digestive disorders and contribute to improved health and vitality of the host animal. As healthy animals utilize and convert nutrients of ingested feeds effectively into steady growth, the beneficial impact of probiotic on the intestinal microbiota can lead to better daily weight gain and feed conversion. Numerous reports have disclosed that an increase in the digestion and absorption of nutrients is a major mechanism responsible for the enhanced growth performance of broilers in response to probiotics supplements (Mountzouris et al., 2010). Moreover, the beneficial probiotic bacteria are recognized to produce digestive enzymes that may help to improve digestion and enhance feed conversion in the host animal. The inconsistency in reports as regards the efficacy of a probiotic on growth performance might be owing to many factors, including variations in rearing conditions, type of strain, and number of viable cells administered, the occurrence of administration of probiotic, and overall diet.

Measurement of internal organ weight is a universal method for the assessment of the immune status of the birds (Heckert, Estevez, Russek-Cohen, & Pettit-Riley, 2002). Several factors that could persuade the size of these organs are related to diet, gut microbiome, and cellular metabolic processes within these organs. In this perspective, it is known that

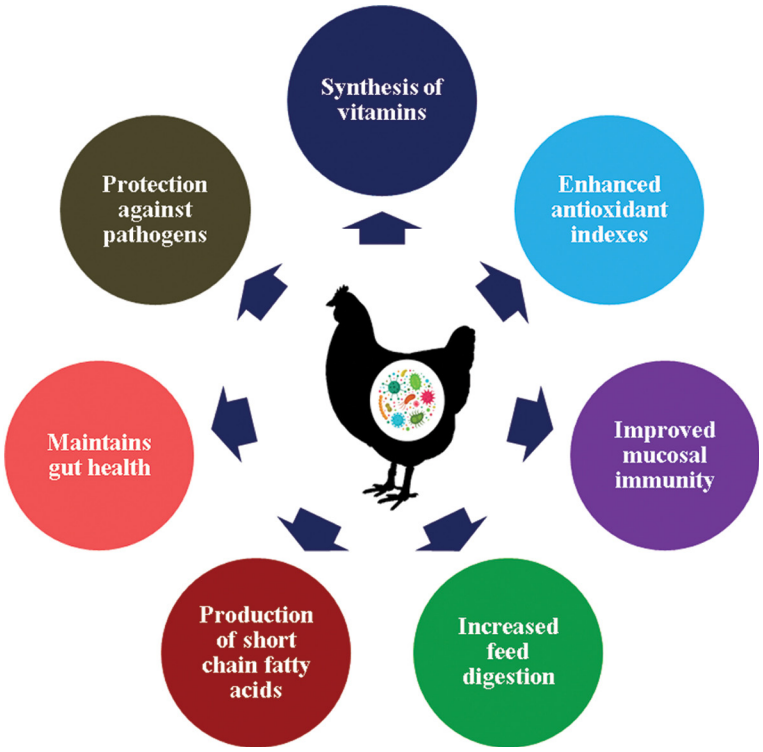


FIGURE 22.2 Key functions of probiotics in poultry.

microbes with probiotic action can colonize the surface of the caecal epithelium during its metabolism, which influences on the augment of the organ size (Chichlowski, Croom, McBride, Havenstein, & Koci, 2007). Bursa of Fabricius is the site of hematopoiesis responsible for B-cell production and plays a crucial role in the immune system of poultry, and the relative weight of bursa might reveal the anatomical response to the immune status of broiler chickens (Willis et al., 2013; Zhang & Kim, 2014) and can also imply the improved immune response. Hussein and Selim (2018) found a relatively greater bursa of Fabricius with dietary inclusion of probiotics. Besides, the bursa of Fabricius is the primary lymphoid and dietary probiotic administration can lead to an increase in weight, which may be considered an improvement of the immune system, though, too much response depress growth performance (Willis et al., 2013). Santoso, Tanaka, Ohtani, and Sakaida (2001) and Agboola, Omidiwura, Odu, Popoola, and Iyayi (2015) documented that dietary supplementation of probiotics may cause lower fat absorption and deposition, which concludes that the probiotic microbe could reduce the activity of acetyl-CoA carboxylase (a key enzyme in fatty acid biosynthesis pathway) and promote fatty acid β-oxidation that lowers the abdominal fat deposition The regulation of lipid metabolism to decrease the abdominal fat content based on dietary components and feeding approach, as well as elucidating their effects on the key enzymes associated with lipid metabolism, might assist the production of lean meat and aid to understand the fat-lowering effects of diet and different feeding strategies (Fouad & El-Senousey, 2014).

Hatab, Elsayed, and Ibrahim (2016) documented that there was a significant reduction in serum level of total cholesterol and triglyceride in broilers fed with the probiotic mixture of *B. subtilis* and *E. faecium*. Furthermore, they exemplify that *B. subtilis* and *E. faecium* have anticholesterolemic effects, influenced fatty acid synthesis in the liver of layer chicks as signified by a reduced activity of acetyl-CoA carboxylase. In similar aspects, Saleh, Hayashi, & Ohtsuka, (2013) and Shokryazdan et al. (2017) reported that *Saccharomyces* and *Lactobacillus* supplement in the diet lowered triglyceride and blood cholesterol levels in broiler chickens. Also, an analogous consequence was attained by Salarmoini and Fooladi (2011); they recorded that *B. subtilis* and *Bacillus licheniformis* were able to produce esterase enzyme together with lipase enzymes, which converts free fatty acid to esterified form triglyceride in intestinal content and lastly fewer possibility for triglyceride absorption into the plasma. The decreased Triglyceride Level (TGL) concentration in carcass would result in better meat for the consumers and also low in hepatic TGL level is recognized to be healthier for broilers by reducing the formation of the fatty liver syndrome.

Furthermore, Salehizadeh, Modarressi, Mousavi, & Ebrahimi (2019) proclaimed that native and commercial *Lactobacillus* caused a significant reduction in cholesterol and triglyceride levels of broilers. They hypothesized that the anticholesterolemic effect of probiotic might be associated to deconjugate the bile salts, as well as obstructed the activity of acetyl-CoA carboxylase and 3-hydroxyl-3-methylglutaryl coenzyme A reductase, which are the rate-limiting enzymes of cholesterogenesis. Consequently, the previous findings substantiate the fact that indigenous probiotic microbes have played a vital role in the reduction of cholesterol level for broilers. At the same time, a contradictory result was also obtained by Owosibo, Odetola, Odunsi, Adejinmi, and Lawrence-Azua (2013), who found a significant increase in the serum cholesterol level by the administration of the probiotics in broiler chicks. Besides, Kawahara, Ueda, and Nomura (1991) described that probiotic microbe did not show any variations in total serum cholesterol value.

Hatab et al. (2016) found a significant increase in calcium concentrations in the probiotic supplemented groups as compared with the control group. Previous studies by Scholz-Ahrens et al. (2007) stated that probiotics can enhance calcium absorption from the GI tract. Several researchers emphasized that probiotics enhance the absorption of minerals like calcium and iron from the GI tract by improving the solubility of minerals and by improving the surface absorption attributable to the propagation of enterocytes (Eizaguirre et al., 2002; Scholz-Ahrens et al., 2007). Conversely, Hashemzadeh, Rahimi, Torshizi, and Masoudi (2013) affirmed no significant influence of probiotic on serum calcium and phosphorus concentrations in broiler chicks.

6 Bacterial populations in GI tract of poultry

The bacterial community of the gut ultimately imitates the coevolution of microorganisms with their host and the diet adopted by the host. In broilers, the diet and environment influence the microbial category of the GI tract. Dirty litter and other managing parameters involve the microbial populations of the chickens both directly by providing an incessant source of bacteria and indirectly by manipulating the physical condition and defense of the chickens (Apajalahti, Kettunen, & Graham, 2004). During the past decades, the probiotics have revealed varying results of its growth-promoting efficacy in broilers (Jin, Ho, Abdullah, Ali, & Jalaludin, 1998; Zulkifli, Norma, Israf, & Omar, 2002; Mountzouris et al., 2010). It is commonly believed that the growth-enhancing effect of probiotics is due to the growth of gut microbiota and their contribution to beneficial functions in the intestine. In chicken, the caecum is a complex ecosystem that comprises a highly varied microbiota.

In, recent decades, the composition of poultry gut microbiota has been broadly studied that divulge the prevalence of bacterial genera such as *Lactobacillus* (*L. casei*, *L. fermentum*, *L. gasseri*, *L. acidophilus*, *L. rhamnosus*, and *L. paracasei*), *Streptococcus*, *Lactococcus*, *Leuconostoc*, and *Bacillus*, etc. Next to bacteria, actinobacteria are one of the imperative gut floras of animals and are well-known to form intimate association with vertebrates and invertebrates. Most of the poultry gut actinobacteria (e.g., *Bifidobacterium*, *Streptomyces*, and *Rhodoccoccus*) are advantageous and have been reported markedly in the works of literature (Kim, Garson, & Fuerst, 2005; Tan, Deng, & Cao, 2009; Wu et al., 2010; Latha et al., 2016). The symbiotic associations of actinobacteria with poultry are requisite for their survival and reproduction since they play a pivotal role in host health by maintaining a well-balanced intestinal microbiota, breakdown of complex nutrients by synthesizing digestive enzymes, producing vitamins and essential amino acids, and detoxification of toxic compounds (Scott et al., 2008; Visser, Nobre, Currie, Aanen, & Poulsen, 2012). To explore potential applications of the huge community of the gut bacteria, numerous studies have been performed to characterize the cultivable animal fecal actinobacteria in turn to assess the diversity of gut actinobacterial community and their biopotential traits. Mara and Oragui (1981) isolated *Micromonospora* spp. and *Streptomyces* spp. from feces of domestic avian species (ducks, geese, and turkeys). Diverse actinobacterial genera have been previously reported in a range of birds like black-headed gull (8 genera), Indian peafowl (18 genera), wreathed hornbill (8 genera), red-crowned crane (9 genera), whooper swan (5 genera), and common ostrich (6 genera). Among those *Streptomyces*, *Rhodococcus* and *Arthrobacter* were the prevalent genera with the widest distribution. Furthermore, their antimicrobial activity results signify that not only the diversity of bird fecal actinobacteria was very complex but also the bioactivity was broad and high (Chen et al., 2014).

7 Application of actinobacteria as probiotics in livestock and poultry production

Lately, the actinobacteria were exploited as probiotics in livestock production, since they possess multifaceted probiotic attributes such as surviving under GI tract conditions, better colonization ability, ability to synthesize bioactive metabolites, and detoxification of toxic compounds. The beneficial effects of the administration of actinobacteria-based probiotics have been shown to extend to livestock animals, though only limited information is available.

Tan et al. (2009) reported that the actinobacterial genera such as *Streptomyces*, *Nocardiopsis*, and *Oerskovia* isolated from feces of healthy goats existing in pastures of Runan Town, Henan Province, China, can be utilized as probiotic for improving animal production due to their noteworthy biopotentialities such as antimicrobial activities against bacterial and fungal pathogens (*E. coli*, *B. subtilis*, *Pseudomonas aeruginosa*, *Candida albicans*, *Fusarium oxysporum*, and *Trichoderma atroviride*), resistance to different heavy metals (chromium, nickel, and copper), and have the capability to synthesize different enzymes exo-enzymes (amylase, protease, cellulase, xylanase, pectin lyase, alginate lyase, α-galactosidase, and β-galactosidase), and their abundance and metabolic diversity recommended a great impact on the digestive function of the host. Latha and Dhanasekaran (2013), isolated probiotic actinobacteria from the feces of chickens and goats and reported a total actinobacterial population density ranging from 2.48 to 6.11 log CFU gfw^{-1} and most of the isolated actinobacteria able to secrete digestive enzymes (amylolytic, proteolytic, and cellulolytic) and exhibited notable antimicrobial activity against *E. coli*, *Salmonella typhi*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Klebsiella* sp., and *Pseudomonas* sp.

Latha et al. (2016) stated that the *Streptomyces* sp. from chicken feces exhibited excellent viability at pH 2 and survived better in the presence of pepsin at 3 mg mL^{-1}, moreover their tolerance with respect to both bile (at 0.3%) and pancreatin (at 1 mg mL^{-1}) imply that strains from animal origin would be better in resistant prevailing harsh conditions of the GI tract. The synthesis of a specialized inhibitory substance called "bacteriocins." *Streptomyces* sp. JD9 from the feces of chicken revealed a multiple inhibitory activity through by production of bacteriocin against the various bacterial pathogens, namely, *E. coli* MTCC 9537, *Salmonella enterica* MTCC 3224, *Shigella flexneri* MTCC 9543, *Klebsiella pneumoniae* MTCC 109, *Enterococcus faecalis* MTCC 439, and *S. aureus* MTCC 96 (Latha & Dhanasekaran, 2015). Water and animal feed are the key routes for heavy metal contamination in the biological systems, and it can accumulate in the body through the consumption of metal-contaminated water and food. Consequently, it is necessary to remove or detoxify these toxic heavy metals to manage the trouble of bioaccumulation in living organisms, mainly in livestock. In this view, Latha & Dhanasekaran (2015) reported that probiotic actinobacterial isolate *Streptomyces werraensis* LD22 showed high tolerance to the heavy metals like $K_2Cr_2O_7$, $NiCl_2$, $ZnCl_2$, $CuSO_4$, and $PbNO_3$ at 250 mg L^{-1} concentration along with 82.3% of biosorption at 100 mg L^{-1} of Cr(VI). In addition, the aggregating ability of actinobacteria was enhanced after the addition of filter-sterilized spent supernatant of probiotic strain *S. werraensis* LD22 by secretion of a heat-stable, acidic pH resistant, low-molecular-weight peptide pheromone (12 kDa) which not only promoted the aggregation propensity but also induced the biofilm-forming ability of other probiotic actinobacterial isolates (Muthu Selvam et al., 2016). On the whole, the above studies indicated that actinobacterial probiont positively contributes to the overall health status of their hosts.

8 Conclusion

The exploitation of probiotics as a feed supplement in broiler production has tremendously increased over the last decade, particularly since the forbid on antibiotic growth promoters in the poultry sector. A range of microorganisms like *Lactic acid bacteria*, *Bacillus*, *Bifidobacteria*, *Enterococcus*, *Streptomyces*, and yeast have been instigated as potential probionts. The beneficial attributes of several developed probiotics have been well disclosed for promising new interferences target specific functions such as prevention of diseases, synthesis of vitamins, digestive enzymes, and short chain fatty acids to stimulate cellular pathways by improving mucosal barrier in systemic effects on poultry health. Moreover, the mode of action of these probiotics needs to be better understood to accomplish the ultimate goal of reducing or eradicating the antibiotic use in the poultry feed as a growth promoter. Therefore, it is a pressing necessity to develop novel probiotics that are virtually more feasible under farm conditions.

References

Abdel-Rahman, M. A., Tashiro, Y., & Sonomoto, K. (2013). Recent advances in lactic acid production by microbial fermentation processes. *Biotechnology Advances*, *31*(6), 877–902.

Agboola, A. F., Omidiwura, B. R. O., Odu, O., Popoola, I. O., & Iyayi, E. A. (2015). Effects of organic acid and probiotic on performance and gut morphology in broiler chickens. *South African Journal of Animal Science*, *45*(5), 494–501.

Ahasan, A. S. M. L., Agazzi, A., Invernizzi, G., Bontempo, V., & Savoini, G. (2015). The beneficial role of probiotics in monogastric animal nutrition and health. *Journal of Dairy, Veterinary & Animal Research*, *2*(4), 00041.

Apajalahti, J., Kettunen, A., & Graham, H. (2004). Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. *World's Poultry Science Journal*, *60*(2), 223–232.

Arévalo-Villena, M., Fernandez-Pacheco, P., Castillo, N., Bevilacqua, A., & Pérez, A. B. (2018). Probiotic capability in yeasts: Set-up of a screening method. *LWT—Food Science and Technology*, *89*, 657–665.

Atela, J. A., Mlambo, V., & Mnisi, C. M. (2019). A multi-strain probiotic administered via drinking water enhances feed conversion efficiency and meat quality traits in indigenous chickens. *Animal Nutrition*, *5*(2), 179–184.

Atterbury, R. J., Hobley, L., Till, R., Lambert, C., Capeness, M. J., Lerner, T. R., et al. (2011). Effects of orally administered *Bdellovibrio bacteriovorus* on the well-being and Salmonella colonization of young chicks. *Applied Environmental Microbiology*, *77*(16), 5794–5803.

Banjara, N., Suhr, M. J., & Hallen-Adams, H. E. (2015). Diversity of yeast and mold species from a variety of cheese types. *Current Microbiology*, *70*(6), 792–800.

Brisbin, J. T., Gong, J., & Sharif, S. (2008). Interactions between commensal bacteria and the gut-associated immune system of the chicken. *Animal Health Research Reviews*, *9*(1), 101–110.

Caracciolo, A. B., Topp, E., & Grenni, P. (2015). Pharmaceuticals in the environment: Biodegradation and effects on natural microbial communities. A review. *Journal of Pharmaceutical and Biomedical Analysis*, *106*, 25–36.

Champagne, C. P., Gardner, N. J., & Roy, D. (2005). Challenges in the addition of probiotic cultures to foods. *Critical Reviews in Food Science and Nutrition*, *45*(1), 61–84.

Chattopadhyay, M. K. (2014). Use of antibiotics as feed additives: A burning question. *Frontiers in Microbiology*, *5*, 334.

Chen, X., Qiu, S., Jiang, Y., Han, L., Huang, X., & Jiang, C. (2014). Diversity, bioactivity and drug development of cultivable actinobacteria in six species of bird feces. *American Journal of BioScience*, *2*(1), 13–18.

Chichlowski, M., Croom, J., McBride, B. W., Havenstein, G. B., & Koci, M. D. (2007). Metabolic and physiological impact of probiotics or direct-fed-microbials on poultry: A brief review of current knowledge. *International Journal of Poultry Science*, *6*(10), 694–704.

Culligan, E. P., Hill, C., & Sleator, R. D. (2009). Probiotics and gastrointestinal disease: Successes, problems and future prospects. *Gut Pathogens*, *1*(1), 1–12.

Cutting, S. M. (2011). Bacillus probiotics. *Food Microbiology*, *28*(2), 214–220.

Dahiya, J. P., Wilkie, D. C., Van Kessel, A. G., & Drew, M. D. (2006). Potential strategies for controlling necrotic enteritis in broiler chickens in post-antibiotic era. *Animal Feed Science and Technology*, *129*(1–2), 60–88.

Danzeisen, J. L., Kim, H. B., Isaacson, R. E., Tu, Z. J., & Johnson, T. J. (2011). Modulations of the chicken cecal microbiome and metagenome in response to anticoccidial and growth promoter treatment. *PLoS One*, *6*(11), e27949.

De Jesus-Laboy, K. M., Godoy-Vitorino, F., Piceno, Y. M., Tom, L. M., Pantoja-Feliciano, I. G., Rivera-Rivera, M. J., et al. (2011). Comparison of the fecal microbiota in feral and domestic goats. *Genes*, *3*(1), 1–18.

de Melo Pereira, G. V., de Oliveira Coelho, B., Júnior, A. I. M., Thomaz-Soccol, V., & Soccol, C. R. (2018). How to select a probiotic? A review and update of methods and criteria. *Biotechnology Advances*, *36*(8), 2060–2076.

Del Re, B., Sgorbati, B., Miglioli, M., & Palenzona, D. (2000). Adhesion, autoaggregation and hydrophobicity of 13 strains of *Bifidobacterium longum*. *Letters in Applied Microbiology*, *31*(6), 438–442.

Ding, C., & He, J. (2010). Effect of antibiotics in the environment on microbial populations. *Applied Microbiology and Biotechnology*, *87*(3), 925–941.

Eizaguirre, I., Urkia, N. G., Asensio, A. B., Zubillaga, I., Zubillaga, P., Vidales, C., et al. (2002). Probiotic supplementation reduces the risk of bacterial translocation in experimental short bowel syndrome. *Journal of Pediatric Surgery*, *37*(5), 699–702.

Emami, N. K., Samie, A., Rahmani, H. R., & Ruiz-Feria, C. A. (2012). The effect of peppermint essential oil and fructooligosaccharides, as alternatives to virginiamycin, on growth performance, digestibility, gut morphology and immune response of male broilers. *Animal Feed Science and Technology*, *175*(1–2), 57–64.

Engberg, R. M., Hedemann, M. S., Leser, T. D., & Jensen, B. B. (2000). Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. *Poultry Science*, *79*(9), 1311–1319.

Fadda, M. E., Mossa, V., Deplano, M., Pisano, M. B., & Cosentino, S. (2017). In vitro screening of Kluyveromyces strains isolated from Fiore Sardo cheese for potential use as probiotics. *LWT—Food Science and Technology*, *75*, 100–106.

FAO/WHO. (2001). *Report of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria*. Cordoba, Argentina.

FAO/WHO. (2009). *How to feed the world in 2050*. Rome, Italy, http://www .fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf.

Foligné, B., Dewulf, J., Vandekerckove, P., Pignède, G., & Pot, B. (2010). Probiotic yeasts: Anti-inflammatory potential of various non-pathogenic strains in experimental colitis in mice. *World Journal of Gastroenterology*, *16*(17), 2134.

Fouad, A. M., & El-Senousey, H. K. (2014). Nutritional factors affecting abdominal fat deposition in poultry: A review. *Asian-Australasian Journal of Animal Sciences*, *27*(7), 1057–1068.

Fleet, G. H. (2006). The commercial and community significance of yeasts in food and beverage production. In A. Querol, & G. H. Fleet (Eds.), *Yeasts in food and beverages* (pp. 1–12). New York: Springer Inc.

Franklin, A. M., Aga, D. S., Cytryn, E., Durso, L. M., McLain, J. E., Pruden, A., et al. (2016). Antibiotics in agroecosystems: Introduction to the special section. *Journal of Environmental Quality*, *45*(2), 377–393.

Fuller, R. (1989). Probiotics in man and animals. *Journal of Applied Bacteriology*, *66*(5), 365–378.

Furtula, V., Farrell, E. G., Diarrassouba, F., Rempel, H., Pritchard, J., & Diarra, M. S. (2010). Veterinary pharmaceuticals and antibiotic resistance of *Escherichia coli* isolates in poultry litter from commercial farms and controlled feeding trials. *Poultry Science*, *89*(1), 180–188.

Gadde, U., Kim, W. H., Oh, S. T., & Lillehoj, H. S. (2017). Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: A review. *Animal Health Research Reviews*, *18*(1), 26–45.

Galopin, S., Cattoir, V., & Leclercq, R. (2009). A chromosomal chloramphenicol acetyltransferase determinant from a probiotic strain of *Bacillus clausii*. *FEMS Microbiology Letters*, *296*(2), 185–189.

Garcia Hernandez, Y., Rodríguez, Z., Brandão, L. R., Rosa, C. A., Nicoli, J. R., Iglesias, A. E., et al. (2012). Identification and in vitro screening of avian yeasts for use as probiotic. *Research in Veterinary Science*, *93*(2), 798–802.

Gaw, S., Thomas, K. V., & Hutchinson, T. H. (2014). Sources, impacts and trends of pharmaceuticals in the marine and coastal environment. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*(1656), 20130572.

Gil-Rodríguez, A. M., Carrascosa, A. V., & Requena, T. (2015). Yeasts in foods and beverages: In vitro characterisation of probiotic traits. *LWT—Food Science and Technology*, *64*(2), 1156–1162.

Goodarzi, A. G. (2016). Obtaining of *Lactobacillus delbrueckii* cold sensitive rif mutants for shelf life prolongation of dairy products. *International Journal of Current Microbiology and Applied Sciences*, *5*(1), 546–552.

Gueimonde, M., Sánchez, B., de los Reyes-Gavilán, C. G., & Margolles, A. (2013). Antibiotic resistance in probiotic bacteria. *Frontiers in Microbiology*, *4*, 1–6.

Hammad, A. M., & Shimamoto, T. (2010). Towards a compatible probiotic-antibiotic combination therapy: Assessment of antimicrobial resistance in the Japanese probiotics. *Journal of Applied Microbiology*, *109*(4), 1349–1360.

Hardy, H., Harris, J., Lyon, E., Beal, J., & Foey, A. D. (2013). Probiotics, prebiotics and immunomodulation of gut mucosal defences: Homeostasis and immunopathology. *Nutrients*, *5*(6), 1869–1912.

Harms, R. H., Ruiz, N., & Miles, R. D. (1986). Influence of virginiamycin on broilers fed four levels of energy. *Poultry Science*, *65*(10), 1984–1986.

Hashemzadeh, F., Rahimi, S., Torshizi, M. A. K., & Masoudi, A. A. (2013). Effects of probiotics and antibiotic supplementation on serum biochemistry and intestinal microflora in broiler chicks. *International Journal of Agriculture and Crop Sciences*, *5*(20), 2394–2398.

Hassanein, S. M., & Soliman, N. K. (2010). Effect of probiotic (*Saccharomyces cerevisiae*) adding to diets on intestinal microflora and performance of hy-line layer hens. *The Journal of American Science*, *6*(11), 159–169.

Hatab, M. H., Elsayed, M. A., & Ibrahim, N. S. (2016). Effect of some biological supplementation on productive performance, physiological and immunological response of layer chicks. *Journal of Radiation Research and Applied Sciences*, *9*(2), 185–192.

Heckert, R. A., Estevez, I., Russek-Cohen, E., & Pettit-Riley, R. (2002). Effects of density and perch availability on the immune status of broilers. *Poultry Science*, *81*(4), 451–457.

Hudson, L. E., McDermott, C. D., Stewart, T. P., Hudson, W. H., Rios, D., Fasken, M. B., et al. (2016). Characterization of the probiotic yeast *Saccharomyces boulardii* in the healthy mucosal immune system. *PLoS One*, *11*(4), 1–21.

Hussein, E., & Selim, S. (2018). Efficacy of yeast and multi-strain probiotic alone or in combination on growth performance, carcass traits, blood biochemical constituents, and meat quality of broiler chickens. *Livestock Science*, *216*, 153–159.

Huyghebaert, G., Ducatelle, R., & Van Immerseel, F. (2011). An update on alternatives to antimicrobial growth promoters for broilers. *The Veterinary Journal*, *187*(2), 182–188.

Iannitti, T., & Palmieri, B. (2010). Therapeutical use of probiotic formulations in clinical practice. *Clinical Nutrition*, *29*(6), 701–725.

Jechalke, S., Heuer, H., Siemens, J., Amelung, W., & Smalla, K. (2014). Fate and effects of veterinary antibiotics in soil. *Trends in Microbiology*, *22*(9), 536–545.

Jiang, T., Li, H. S., Han, G. G., Singh, B., Kang, S. K., Bok, J. D., et al. (2017). Oral delivery of probiotics in poultry using pH-sensitive tablets. *Journal of Microbiology and Biotechnology*, *27*(4), 739–746.

Jin, L. Z., Ho, Y. W., Abdullah, N., Ali, M. A., & Jalaludin, S. (1998). Effects of adherent *Lactobacillus* cultures on growth, weight of organs and intestinal microflora and volatile fatty acids in broilers. *Animal Feed Science and Technology*, *70*(3), 197–209.

Kawahara, E., Ueda, T., & Nomura, S. (1991). In vitro phagocytic activity of white-spotted char blood cells after injection with *Aeromonas salmonicida* extracellular products. *Fish Pathology*, *26*(4), 213–214.

Keen, P. L., & Patrick, D. M. (2013). Tracking change: A look at the ecological footprint of antibiotics and antimicrobial resistance. *Antibiotics*, *2*(2), 191–205.

Kerr, J. R. (1999). Bacterial inhibition of fungal growth and pathogenicity. *Microbial Ecology in Health and Disease*, *11*(3), 129–142.

Khan, R. U., & Naz, S. (2013). The applications of probiotics in poultry production. *World's Poultry Science Journal*, *69*(3), 621–632.

Khochamit, N., Siripornadulsil, S., Sukon, P., & Siripornadulsil, W. (2015). Antibacterial activity and genotypic–phenotypic characteristics of bacteriocin-producing *Bacillus subtilis* KKU213: Potential as a probiotic strain. *Microbiological Research*, *170*, 36–50.

Kim, T. K., Garson, M. J., & Fuerst, J. A. (2005). Marine actinomycetes related to the 'Salinospora' group from the Great Barrier Reef sponge *Pseudoceratina clavata*. *Environmental Microbiology*, *7*(4), 509–518.

Kong, Y., Teather, R., & Forster, R. (2010). Composition, spatial distribution, and diversity of the bacterial communities in the rumen of cows fed different forages. *FEMS Microbiology Ecology*, *74*(3), 612–622.

Kral, M., Angelovicova, M., Mrazova, L., Tkacova, J., & Kliment, M. (2011). Probiotic and acetic acid effect on broiler chickens performance. *Scientific Papers Animal Science and Biotechnologies*, *44*(1), 62–64.

Kumar, K., Gupta, S. C., Baidoo, S. K., Chander, Y., & Rosen, C. J. (2005). Antibiotic uptake by plants from soil fertilized with animal manure. *Journal of Environmental Quality*, *34*(6), 2082–2085.

Kümmerer, K. (2009). Antibiotics in the aquatic environment—A review—Part I. *Chemosphere*, *75*(4), 417–434.

Lane, M. M., & Morrissey, J. P. (2010). *Kluyveromyces marxianus*: A yeast emerging from its sister's shadow. *Fungal Biology Reviews*, *24*(1–2), 17–26.

Latha, S., & Dhanasekaran, D. (2013). Antibacterial and extracellular enzyme activities of gut actinobacteria isolated from *Gallus gallus* domesticus and *Capra hircus*. *Journal of Chemical and Pharmaceutical Research*, *5*(11), 379–385.

Latha, S., & Dhanasekaran, D. (2015). Bacteriocin: A natural alternative to synthetic antibacterial antibiotics. In *Antimicrobials* (pp. 172–191). Boca Raton, FL, USA: CRC Press, Tailor & Francis Group.

Latha, S., Vinothini, G., Calvin, D. J. D., & Dhanasekaran, D. (2016). In vitro probiotic profile based selection of indigenous actinobacterial probiont *Streptomyces* sp. JD9 for enhanced broiler production. *Journal of Bioscience and Bioengineering*, *121*(1), 124–131.

Lee, K. W., Hong, Y. H., Lee, S. H., Jang, S. I., Park, M. S., Bautista, D. A., et al. (2012). Effects of anticoccidial and antibiotic growth promoter programs on broiler performance and immune status. *Research in Veterinary Science*, *93*(2), 721–728.

Lilly, D. M., & Stillwell, R. H. (1965). Probiotics: Growth-promoting factors produced by microorganisms. *Science*, *147*(3659), 747–748.

Lin, J., Hunkapiller, A. A., Layton, A. C., Chang, Y. J., & Robbins, K. R. (2013). Response of intestinal microbiota to antibiotic growth promoters in chickens. *Foodborne Pathogens and Disease*, *10*(4), 331–337.

Liu, C. H., Wu, K., Chu, T. W., & Wu, T. M. (2018). Dietary supplementation of probiotic, *Bacillus subtilis* E20, enhances the growth performance and disease resistance against *Vibrio alginolyticus* in parrot fish (*Oplegnathus fasciatus*). *Aquaculture International*, *26*(1), 63–74.

Mara, D. D., & Oragui, J. I. (1981). Occurrence of *Rhodococcus coprophilus* and associated actinomycetes in feces, sewage, and freshwater. *Applied and Environmental Microbiology*, *42*(6), 1037–1042.

Markowiak, P., & Śliżewska, K. (2018). The role of probiotics, prebiotics and synbiotics in animal nutrition. *Gut Pathogens*, *10*(1), 21.

Mehdi, Y., Létourneau-Montminy, M. P., Gaucher, M. L., Chorfi, Y., Suresh, G., Rouissi, T., et al. (2018). Use of antibiotics in broiler production: Global impacts and alternatives. *Animal Nutrition*, *4*(2), 170–178.

Metchnikoff, E. (1907). Lactic acid as inhibiting intestinal putrefaction. In P. C. Mitchell (Ed.), *The prolongation of life: Optimistic studies* (pp. 161–183). London: Heinemann.

Mookiah, S., Sieo, C. C., Ramasamy, K., Abdullah, N., & Ho, Y. W. (2014). Effects of dietary prebiotics, probiotic and synbiotics on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens. *Journal of the Science of Food and Agriculture*, *94*(2), 341–348.

Mountzouris, K. C., Tsitrsikos, P., Palamidi, I., Arvaniti, A., Mohnl, M., Schatzmayr, G., et al. (2010). Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition. *Poultry Science*, *89*(1), 58–67.

Muthu Selvam, R., Vinothini, G., Palliyarai Thaiyammal, S., Latha, S., Chinnathambi, A., Dhanasekaran, D., et al. (2016). The cell aggregating propensity of probiotic actinobacterial isolates: Isolation and characterization of the aggregation inducing peptide pheromone. *Biofouling*, *32*(1), 71–79.

Nissle, A. (1959). Explanations of the significance of colonic dysbacteria & the mechanism of action of *E. coli* therapy (Mutaflor). *Die Medizinische*, *4*(21), 1017–1022.

Nunes, J. O., Bertechini, A. G., de Brito, J. A. G., Fassani, E. J., Mesquita, F. R., Makiyama, L., et al. (2012). Evaluation of the use of probiotic (*Bacillus subtilis* C-3102) as additive to improve performance in broiler chicken diets. *Revista Brasileira de Zootecnia*, *41*(11), 2374–2378.

Owosibo, A. O., Odetola, O. M., Odunsi, O. O., Adejinmi, O. O., & Lawrence-Azua, O. O. (2013). Growth, haematology and serum biochemistry of broilers fed probiotics based diets. *African Journal of Agricultural*, *8*(41), 5076–5081.

O'Brien, T. F. (2002). Emergence, spread, and environmental effect of antimicrobial resistance: How use of an antimicrobial anywhere can increase resistance to any antimicrobial anywhere else. *Clinical Infectious Diseases*, *34*(Suppl. 3), S78–S84.

Parker, R. B. (1974). Probiotics, the other half of the antibiotic story. *Animal Nutrition & Health*, *29*(29), 4–8.

Peric, L., Milosevic, N., Zikic, D., Bjedov, S., Cvetkovic, D., Markov, S., et al. (2010). Effects of probiotic and phytogenic products on performance, gut morphology and cecal microflora of broiler chickens. *Archiv Tierzucht*, *53*(3), 350–359.

Picard, C., Fioramonti, J., Francois, A., Robinson, T., Neant, F., & Matuchansky, C. (2005). Review article: Bifidobacteria as probiotic agents—Physiological effects and clinical benefits. *Alimentary Pharmacology & Therapeutics*, *22*(6), 495–512.

Popova, T. (2017). Effect of probiotics in poultry for improving meat quality. *Current Opinion in Food Science*, *14*, 72–77.

Ranadheera, C. S., Evans, C. A., Adams, M. C., & Baines, S. K. (2012). Probiotic viability and physico-chemical and sensory properties of plain and stirred fruit yogurts made from goat's milk. *Food Chemistry*, *135*(3), 1411–1418.

Ripert, G., Racedo, S. M., Elie, A. M., Jacquot, C., Bressollier, P., & Urdaci, M. C. (2016). Secreted compounds of the probiotic *Bacillus clausii* strain O/C inhibit the cytotoxic effects induced by *Clostridium difficile* and *Bacillus cereus* toxins. *Antimicrobial Agents and Chemotherapy*, *60*(6), 3445–3454.

Russell, D. A., Ross, R. P., Fitzgerald, G. F., & Stanton, C. (2011). Metabolic activities and probiotic potential of bifidobacteria. *International Journal of Food Microbiology*, *149*(1), 88–105.

Saarela, M., Mogensen, G., Fonden, R., Mättö, J., & Mattila-Sandholm, T. (2000). Probiotic bacteria: Safety, functional and technological properties. *Journal of Biotechnology*, *84*(3), 197–215.

Salarmoini, M., & Fooladi, M. H. (2011). Efficacy of *Lactobacillus acidophilus* as probiotic to improve broiler chicks performance. *Journal of Agricultural Science and Technology*, *13*(2), 165–172.

Saleh, A. A., Hayashi, K., & Ohtsuka, A. (2013). Synergistic effect of feeding *Aspergillus awamori* and *Saccharomyces cerevisiae* on growth performance in broiler chickens; promotion of protein metabolism and modification of fatty acid profile in the muscle. *The Journal of Poultry Science*, *50*(3), 242–250.

Salehizadeh, M., Modarressi, M. H., Mousavi, S. N., & Ebrahimi, M. T. (2019). Effects of probiotic lactic acid bacteria on growth performance, carcass characteristics, hematological indices, humoral immunity, and IGF-I gene expression in broiler chicken. *Tropical Animal Health and Production*, *51*(8), 2279–2286.

Salminen, S. (1996). Uniqueness of probiotic strains. *Newsletter-International Dairy Federation*, 18.

Sanders, M. E., Akkermans, L. M., Haller, D., Hammerman, C., Heimbach, J. T., Hörmannsperger, G., et al. (2010). Safety assessment of probiotics for human use. *Gut Microbes*, *1*(3), 164–185.

Sanders, M. E., Shane, A. L., & Merenstein, D. J. (2016). Advancing probiotic research in humans in the United States: Challenges and strategies. *Gut Microbes*, *7*(2), 97–100.

Santoso, U., Tanaka, K., Ohtani, S., & Sakaida, M. (2001). Effect of fermented product from *Bacillus subtilis* on feed conversion efficiency, lipid accumulation and ammonia production in broiler chicks. *Asian-Australasian Journal of Animal Sciences*, *14*(3), 333–337.

Scholz-Ahrens, K. E., Ade, P., Marten, B., Weber, P., Timm, W., Aζil, Y., et al. (2007). Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. *The Journal of Nutrition*, *137*(3), 838S–846S.

Scott, J. J., Oh, D. C., Yuceer, M. C., Klepzig, K. D., Clardy, J., & Currie, C. R. (2008). Bacterial protection of beetle-fungus mutualism. *Science*, *322*(5898), 63.

Seal, B. S., Lillehoj, H. S., Donovan, D. M., & Gay, C. G. (2013). Alternatives to antibiotics: A symposium on the challenges and solutions for animal production. *Animal Health Research Reviews*, *14*(1), 78–87.

Seifi, S., Shirzad, M. R., & Habibi, H. (2013). Effects of probiotic yoghurt and prebiotic utilization on performance and some haematological parameters in broiler chickens. *Acta Scientiae Veterinariae*, *41*(1), 1–6.

Senan, S., Prajapati, J. B., & Joshi, C. G. (2015). Feasibility of genome-wide screening for biosafety assessment of probiotics: A case study of *Lactobacillus helveticus* MTCC 5463. *Probiotics and Antimicrobial Proteins*, *7*(4), 249–258.

Shirota, M., Aso, K., & Iwabuchi, A. (1966). Studies on intestinal microflora. 1. Its constitution in healthy infants and the effect of oral administration of *L. acidophilus* strain Shirota. *Nihon Saikingaku Zasshi [Japanese Journal of Bacteriology]*, *21*(5), 274–283.

Shokryazdan, P., Jahromi, M. F., Liang, J. B., Ramasamy, K., Sieo, C. C., & Ho, Y. W. (2017). Effects of a *Lactobacillus salivarius* mixture on performance, intestinal health and serum lipids of broiler chickens. *PLoS One*, *12*(5), e0175959.

Smirnov, A., Perez, R., Amit-Romach, E., Sklan, D., & Uni, Z. (2005). Mucin dynamics and microbial populations in chicken small intestine are changed by dietary probiotic and antibiotic growth promoter supplementation. *The Journal of Nutrition*, *135*(2), 187–192.

Sood, U., Gupta, V., Kumar, R., Lal, S., Fawcett, D., Rattan, S., et al. (2019). Chicken gut microbiome and human health: Past scenarios, current perspectives, and futuristic applications. *Indian Journal of Microbiology*, *60*(1), 2–11.

Suresh, G., Das, R. K., Kaur Brar, S., Rouissi, T., Avalos Ramirez, A., Chorfi, Y., et al. (2018). Alternatives to antibiotics in poultry feed: Molecular perspectives. *Critical Reviews in Microbiology*, *44*(3), 318–335.

Syngai, G. G., Gopi, R., Bharali, R., Dey, S., Lakshmanan, G. A., & Ahmed, G. (2016). Probiotics—The versatile functional food ingredients. *Journal of Food Science and Technology*, *53*(2), 921–933.

Taklimi, S. M. S. M., Lotfollahian, H., Shahne, A. Z., Mirzaei, F., & Alinejad, A. (2012). Study on efficacy of probiotic in broiler chickens diet. *Agricultural Sciences*, *3*(1), 5–8.

Tan, H., Deng, Z., & Cao, L. (2009). Isolation and characterization of actinomycetes from healthy goat faeces. *Letters in Applied Microbiology*, *49*(2), 248–253.

Tasho, R. P., & Cho, J. Y. (2016). Veterinary antibiotics in animal waste, its distribution in soil and uptake by plants: A review. *Science of the Total Environment*, *563*, 366–376.

Teo, A. Y. L., & Tan, H. M. (2005). Inhibition of *Clostridium perfringens* by a novel strain of *Bacillus subtilis* isolated from the gastrointestinal tracts of healthy chickens. *Applied Environmental Microbiology*, *71*(8), 4185–4190.

Tissier, H. (1906). The treatment of intestinal infections by the method of transformation of bacterial intestinal flora. *Critical Reviews of Social Biology*, *60*, 359–361.

Tokuhiro, K., Ishida, N., Kondo, A., & Takahashi, H. (2008). Lactic fermentation of cellobiose by a yeast strain displaying β-glucosidase on the cell surface. *Applied Microbiology and Biotechnology*, *79*(3), 481.

Torok, V. A., Allison, G. E., Percy, N. J., Ophel-Keller, K., & Hughes, R. J. (2011). Influence of antimicrobial feed additives on broiler commensal post-hatch gut microbiota development and performance. *Applied Environmental Microbiology*, *77*(10), 3380–3390.

Verschuere, L., Rombaut, G., Sorgeloos, P., & Verstraete, W. (2000). Probiotic bacteria as biological control agents in aquaculture. *Microbiology and Molecular Biology Reviews*, *64*(4), 655–671.

Visser, A. A., Nobre, T., Currie, C. R., Aanen, D. K., & Poulsen, M. (2012). Exploring the potential for actinobacteria as defensive symbionts in fungus-growing termites. *Microbial Ecology*, *63*(4), 975–985.

Wierup, M. (2000). The control of microbial diseases in animals: Alternatives to the use of antibiotics. *International Journal of Antimicrobial Agents*, *14*(4), 315–319.

Willis, W. L., Wall, D. C., Isikhuemhen, O. S., Jackson, J. N., Ibrahim, S., Hurley, S. L., et al. (2013). Effect of level and type of mushroom on performance, blood parameters and natural coccidiosis infection in floor-reared broilers. *The Open Mycology Journal*, *7*, 1–6.

Wu, S., Gao, T., Zheng, Y., Wang, W., Cheng, Y., & Wang, G. (2010). Microbial diversity of intestinal contents and mucus in yellow catfish (*Pelteobagrus fulvidraco*). *Aquaculture*, *303*(1), 1–7.

Yang, Q., Zhang, J., Zhu, K., & Zhang, H. (2009). Influence of oxytetracycline on the structure and activity of microbial community in wheat rhizosphere soil. *Journal of Environmental Sciences*, *21*(7), 954–959.

Yegani, M., & Korver, D. R. (2008). Factors affecting intestinal health in poultry. *Poultry Science*, *87*(10), 2052–2063.

Zommiti, M., Chikindas, M. L., & Ferchichi, M. (2019). Probiotics—Live biotherapeutics: A story of success, limitations, and future prospects—Not only for humans. *Probiotics and Antimicrobial Proteins*, *12*, 1266–1289.

Zhang, Z. F., & Kim, I. H. (2014). Effects of multistrain probiotics on growth performance, apparent ileal nutrient digestibility, blood characteristics, cecal microbial shedding, and excreta odor contents in broilers. *Poultry Science*, *93*(2), 364–370.

Zhang, Z. Y., Liu, C., Zhu, Y. Z., Zhong, Y., Zhu, Y. Q., Zheng, H. J., et al. (2009). Complete genome sequence of *Lactobacillus plantarum* JDM1. *Journal of Bacteriology*, *191*(15), 5020–5021.

Zhang, L., Zhang, L., Zeng, X., Zhou, L., Cao, G., & Yang, C. (2016). Effects of dietary supplementation of probiotic, *Clostridium butyricum*, on growth performance, immune response, intestinal barrier function, and digestive enzyme activity in broiler chickens challenged with *Escherichia coli* K88. *Journal of Animal Science and Biotechnology*, *7*(1), 3.

Zhou, H., Gong, J., Brisbin, J. T., Yu, H., Sanei, B., Sabour, P., et al. (2007). Appropriate chicken sample size for identifying the composition of broiler intestinal microbiota affected by dietary antibiotics, using the polymerase chain reaction-denaturing gradient gel electrophoresis technique. *Poultry Science*, *86*(12), 2541–2549.

Zhou, X., Wang, Y., Gu, Q., & Li, W. (2010). Effect of dietary probiotic, *Bacillus coagulans*, on growth performance, chemical composition, and meat quality of Guangxi Yellow chicken. *Poultry Science*, *89*(3), 588–593.

Zulkifli, I., Norma, M. T. C., Israf, D. A., & Omar, A. R. (2002). The effect of early-age food restriction on heat shock protein 70 response in heat-stressed female broiler chickens. *British Poultry Science*, *43*(1), 141–145.

Chapter 23

Small- and Large-Scale Production of Probiotic Foods, Probiotic Potential and Nutritional Benefits

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1 Introduction

Nowadays, consumers are becoming more health conscious and concerned about beneficial value of food, thus directing manufacturers to emphasize the promotion and production of functional foods to meet the demands of the end consumer. Therefore the key for successful marketing and acceptance of novel foods depends not only on the concept of food quality throughout the chain but also on added value of food functionalities. These novel functional food products are either natural or processed foods that have been fortified with active compounds of known biological activity. These compounds when administered in defined quantities confer various health benefits. The process of food fermentation can be defined as "a controlled microbial growth and enzymatic conversions of major and minor food components" (Marco et al., 2017a). Fermentation is an ancient and traditional way of food preservation techniques that has been followed from centuries together. The history of food fermentation dates back to several centuries that evolved with the evolution of mankind that played a dual role of food preservation and sustaining nutrition to combat hidden hunger (Ray et al., 2016). Historically, the idea of fermentation originated somewhere in between 7000 and 8000 BCE in the areas of Indus Valley (Dani & Masson, 1996). It was established that food fermentation formed an important part of the usual practices of the ancient civilizations as archeologists excavated huge amounts of utensils used for food fermentation. Ancient human civilizations not only the Harappans but also the Greeks, Egyptians, and Romans prepared various fermented products like leavened breads, alcoholic beverages, and fermented milk products that formed an important part of their daily routine. Beverages in particular like wines were introduced around 8500–4000 BCE; however, milk-based fermented beverages have been introduced in the Indian subcontinent in between 6000 BCE. Around the globe, food fermentation is practiced in several countries like Europe and Middle East; however, in India, fermented foods and beverages are an integral and exceptional part of the traditional cultural heritage and ethos. It was until recently in the 18th-century researchers around the world were able to formulate new processes and parameters to decipher the microbial processes responsible for food fermentation. Gay Lussac is credited with important contribution in alcoholic fermentation, whereas Louis Pasteur elucidated the extensive role of microbes in the process of fermentation and often described fermentation as "a living process." While previously the main application of food fermentation was preservation of foods, with the evolution of scientific knowledge, enhancing food quality and improving the organoleptic properties became the main domains (Arroyo-Lopez, Perez-Traves, Querol, & Barrio, 2011). Fermented foods usually comprise those food materials that may include beverages as well that are made through controlled microbial growth and enzymatic conversions of major and minor food components usually carbohydrates. Food fermentation involves the growth of variable species of microbial communities with diverse functions that add to the preservation of the foods and increase palatability. With the advancement of lifestyle and technology from the last few decades, humans have optimized different growth conditions to manipulate the progression of only specific

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00023-X
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types of microbial communities during the process of fermentation to meet the demands of the end consumer. The selected microbial communities in different food ecosystems determine the safety and simultaneously add to the flavor, modify the texture, and increase the palatability with simultaneous increase in aroma as well flavors. Major fermented foods around the globe can be categorized in different categories based on the types of raw materials used, different techniques, varying process variables like temperature, salinity, moisture, etc. On the basis of process parameters and microbial community involved food fermentation can be categorized as alcohol and carbon dioxide (yeast), acetic acid (Acetobacter), lactic acid [lactic acid bacteria (LAB) belonging to genera such as *Leuconostoc*, *Lactobacillus*, and *Streptococcus*], *propionic acid* (*Propionibacterium freudenreichii*), and ammonia and fatty acids (Bacillus, molds). While, on the basis of raw materials, required fermentation may involve the use of meats and fish, dairy, vegetables, soybeans and other legumes, cereals, starchy roots, and grapes and other fruits, various fermented foods around the world form an important part of the traditional cuisine. Major fermented foods that have claimed health benefits can be categorized as fruit and vegetable based such as pickles, sauerkraut, kimchi; dairy based like cheese, yoghurt, kefir; cereal based such as idli, dosa, appam, tempeh; and some beverages such as various wines, kombucha, and vinegar. Due to a significant health impact as suggested by various research groups, these fermented foods have been placed in the national dietary recommendation lists (Swain, Anandharaj, Ray, & Rani, 2014). Beyond the use of fermentation as a major tool for food preservation, it is now a based fact that majority of these fermented foods attribute to diverse health benefits. Probiotic microorganisms that form an important part of the fermentation process provide additional benefits that are at par with the basic nutrition. Myriad studies on human physiology have supported this fact that there are positive relations between fermented food consumption and weight maintenance, reduction in the risk of cardiovascular disease (CVD), type 2 diabetes (T2D), and overall mortality as a result of frequent fermented food consumption (Tapsell, 2015). Individually different foods contribute to different physiological responses individually like fermented milk consumption improves glucose metabolism and reduces muscle soreness induced by acute resistance exercise (Iwasa et al., 2013). Similarly, evidence is accumulating for antidiabetic and antiobesity benefits of kimchi (Lee et al., 2015). In inflammatory bowel diseases and other immune-related pathologies such as arthritis and sclerosis, health benefits of fermented foods have also been proposed, although clinical data have not yet been reported (Lorea Baroja, Kirjavainen, Hekmat, & Reid, 2007). Lastly, although the microbiota–gut–brain axis is a nascent field of research, there is an indication that fermented food consumption can alter mood and brain activity.

2 Role of probiotics in food fermentation

The majority of probiotic foods are fermented foods that consist of enumerable microorganisms that result in the formation of specific types of end products characteristic to the type of predominant type of microorganisms present. Food fermentation is mostly categorized into alcoholic and acidic fermentation wherein LAB with *Lactobacillus*, *Leuconostoc*, *Lactococcus*, and *Streptococcus* being the most common starter cultures.

LAB are involved in a number of different food fermentation processes like production of fermented dairy and alcoholic beverages wherein they work in a close combination with other microbial communities preferably *Lactobacillus* and bifidobacteria resulting in the production of a vast group of compounds such as acetate, lactate, butyrate, and propionate plus the release of short-chain fatty acids with further decrease of pH associated with favorable alteration in the gastrointestinal microecology (Parvez, Malik, Ah Kang, & Kim, 2006). Mostly the firmicutes enhance the food quality of cereal-based fermented food products in a number of ways, that is, by concentrating nutrient profile (release of vitamins and essential amino acids) and slowing down the concentration of antinutrient compounds that are an important group of compounds in cereals, enhancing the sensory qualities (taste, aroma, texture, and appearance) and in addition making the cooking process much easier by reducing cooking times and last but not least preserving the food commodities for longer time period (Anderson & Gilliland, 1999). A vast group of organisms belonging to various phyla are associated with acidic fermentations leading to the production of fermented beverages, most of which are from *Acetobacter*, *Gluconobacter*, and *Gluconacetobacter* genera. Microbial populations involved in spontaneous cocoa fermentations demonstrate yeast–bacterial interactions, with well-defined and interdependent role. These fermentations are dominated by yeasts and both lactic acid and acetic acid bacteria, in a definite succession. Yeasts (mostly *Saccharomyces* sp. and *Candida* sp.) proliferate early, leading to production of ethanol, secretion of pectinolytic enzymes, and metabolization of citric acid, raising the pH. This is followed by a phase in which bacteria predominate; principally the microaerophilic LAB and the ethanol oxidative acetic acid bacteria (Tamime, Saarela, Korslund-Søndergaard, Mistry, & Shah, 2005). Among the different fermentation processes, dairy fermentation leads to the production of the majority of products with vast variation in organoleptic properties. The microbial communities involved in the fermentation of different dairy products are from phylum *Actinobacteria*, *Bifidobacterium*, *Corynebacterium glutamicum*, and *Propionibacterium* (Eales et al., 2017). The role of yeasts in the production of fermented foods is well established. A study revealed that industrial yeasts are genetically and phenotypically distinct from

wild strains and stem from a limited set of ancestral strains adapted to man-made environments (clades of Asian strains such as sake yeasts, one mostly containing wine yeasts, a mixed clade containing bread and other yeasts, and two separate families of beer yeasts). Spontaneous fermentation of grape juice is driven by a complex community of microorganisms. The yeast succession from *Hanseniaspora* to *Saccharomyces* has been established, due to differences on species sensitivity to the produced ethanol, and so, population driven on selectivity (Zeng, Pan, & Guo, 2010).

3 Production of probiotic foods

With the evolution of modern technology, consumer health has always been put at the forefront emphasizing the promotion of healthy and safe foods. From the last two to three decades, there has been a paradigm shift in the consumer behavior for safe, nutritious, and healthy foods. Therefore, with each passing day, more thrust has to be put on the development of novel food products that have added value in a term described as functional foods. Probiotic foods play an important role as far as the development of functional foods with high biological activity is concerned. Probiotic foods contain a myriad type of bioactive compounds as a result of microbial activity which when administered in a definite quantity possesses various health benefits beyond adequate nutrition. Due to these claimed health benefits besides having a traditional tinge probiotic aka fermented foods that may be either produced naturally or fortified with probiotics (Tamang, 2012), this new trend has led various manufacturers and industries to develop these third-generation foods. A number of probiotic foods are being prepared traditionally at household level and are basically indigenous to a particular region or country. Basic nomenclature of majority of the products is derived from the country of origin like Zeer, Kad, Zabady, Laban, Rayeb, and Shubat from Middle East and North Africa; Roub from Sudan; Ergo from Ethiopia; Amasi from Zimbabwe; Chhurpi, Mohi, Philu, Somar, and Shoyu from Himalayan region; idli, dosa, appam, etc. from South India; kimchi from Korea; and filmjölk and långfl from Sweden and various others. Different microbial communities are mostly involved in the production of the fermented food products like acidified milk, kefir, cheese, yoghurt, dahi, creams, etc., and nondairy products that may be fruit- and vegetable-based like pickles, sauerkraut, kimchi, cereal based like idli, dosa, appam, tempeh, miso, etc. For any food system to be called functional or probiotic, the number of viable cell count should be in the range of 108–109 CFU/day depending on the microbial community and the physiological conditions of the host. For labeling purpose a probiotic food must contain a minimum live cell count of at least 106 CFU/g (Borresen et al., 2012). For conferring a progressive physiological impact on human health, a suggested daily intake of 108–109 CFU probiotics per gram food is recommended that sums approximately to 100 g/day of probiotic products per day to deliver the required number of probiotic cells to the human body (Ogue-Bon et al., 2011).

4 Major probiotic foods

4.1 Kimchi

Kimchi derives its meaning from Chinese word “chimchae” that means salted vegetables. The history and origin of kimchi date back to at least CE 100. From various authentic literatures, it has been established that consumption of kimchi and its preparation process has evolved from over CE 400, and traditionally kimchi forms an important vegetable product of Korea and has been listed as a heritage product in 2013 and has a long history of producing kimchi both at household and small-scale level. In Korea on an average per individual consumes 50–200 g of kimchi per day. The kimchi industry has gained pace in Korea from last few decades. Korea individually accounts for more than 1.5 million kimchi consumption that shares a market value of almost 230 million USD. The trend of kimchi consumption has shown a steeping rise in other countries also such as China, Russia, Japan, and the United States and due to a myriad variation in raw materials, mode of preparation, and introduction in other regions of the world, it has entered in the list of global foods and is served in many Korean restaurants around the world. Tons of kimchi are being exported to various countries, the majority of which lie in South and Central Asia. Kimchi is such an important food to the nation’s culture and cuisine that a dehydrated kimchi preparation, the so-called space kimchi, was sent into space with the first Korean astronaut (Song, In, Lim, & Rahim, 2017). Around the globe, and particularly in Korea, hundreds of varieties of Kimchi are prepared by the use of fresh vegetables particularly cabbage-based radish being used as raw materials. The special type of cabbage used for kimchi preparation is called *baechu* cabbage with the addition of red pepper and a variety of spices added for flavor development. With the use of cabbage as the basic raw material, many other fresh vegetables are also used which may include radish, leak, mustard, onions, and cucumbers (Cheigh & Park, 1994). The *baechu* cabbage (Chinese cabbage) has been used as a basic raw material for kimchi preparation since ancient times almost from CE 3rd or 4th century, and the kimchi prepared from *baechu* cabbage is called as “*baechu* kimchi” and forms an important heritage food of the Korean diet.

With the advancement of process parameters involved in the production of Kimchi, various variations in the choice of raw materials, regional discrepancies, and seasonal conditions can be incorporated that has till date led to the production of almost 200 different types of kimchi. The most common type of kimchi produced and consumed in Korea and other parts of the globe is *baechu* kimchi that contributes to almost 70% of the total kimchi. Generally, "*baechu* kimchi" is prepared by lactic acid fermentation of baechu cabbage with the addition of other ingredients like onion, pepper, spices that mainly include garlic and ginger. With each passing day, newer variations in the recipes of kimchi preparations are included, which leads to quality improvement and enhancement of functionality (Kil, 2004). Various studies have shown the effect of addition of different ingredients that increase the nutraceutical and functional value of kimchi, for example, addition of mustard leaf, Chinese pepper, and Korean mistletoe extract leads to an increase in the anticancer potential of kimchi (Kim, 2007; Han et al., 2009). In addition to this, various studies have positively studies the biochemical profile and functional properties of kimchi and revealed that kimchi forms a sink of all the important components of important vitamins (vitamin C, vitamin K, vitamin D, etc.), minerals (K, Ca, Na, etc.), dietary fiber, and pigments that contribute to anticancer, antioxidative, antiatherosclerotic, antidiabetic, and antiobesity effects (Park & Rhee, 1995; Islam & Choi, 2009). These functional characteristics of kimchi have led to an increase in the commercial market size for the consumption of kimchi in both Korean markets and across the globe.

4.2 Production of kimchi

Baechu kimchi is the most important representative form of kimchi preferably consumed across Korea. The preparation process of baechu kimchi involves step-by-step approach starting from the preparation of the baechu kimchi as the basic raw material for production. At first, the cabbage is washed thoroughly under tap water, trimmed, sliced into thinner pieces added with salt and kept for almost 24 hours. After the brining process is complete, the cabbage is drained to remove excess water and different ingredients like garlic, ginger paste, spices that include red pepper and anchovy, etc. are added in ground form. Mostly in the preparation of baechu kimchi, the subingredients are wrapped layer by layer under the cabbage leaves (Cho, 1999). Normally, the standard methods of kimchi preparation are as follows: roughly baechu cabbage (100%) is mixed with 13% sliced radish, 2% green onion, 3.5% red pepper powder, 1.4% garlic, and 0.6% ginger followed by setting up the final salt concentration upto 2.5%. The process parameters, concentration of the subingredients, and addition of new ingredients besides the raw materials vary depending upon geographical variations, economic profile, taste of the local population, and the availability of the required ingredients in that particular area. After the stuffing is complete the cabbage leaves are tightly packaged and incubated at lower temperatures to yield a product of desired quality. Modification in the process parameters like temperatures, time of incubation, anaerobic conditions, vessel type, etc. can be varied to change product functionality and taste. Across Korea, kimchi is prepared usually on the onset of winter when the cabbage production is in bulk and is packed and stored in large earthen vessels to be consumed later in the coming spring season. Different recipes were developed to increase the functional capacity of kimchi, for example, enhancing the anticancer properties of kimchi by mustard leaf addition (Kil, 2004).

4.3 Probiotic value of kimchi

The production of kimchi involves the use of a variety of microorganisms to yield a nutritious product with traditional flavor and health benefiting properties. Different types of microbial communities are involved in kimchi fermentation, most of which are LAB. LAB are the main players involved in the production of organic acids from the lactic acid fermentation of starch that reduces the pH of the medium. In addition to the production of lactic acid, various other organic acids like butyric acid, linoleic acids, certain bacteriocins, and alcohols are produced in the due course of fermentation. Generally, LAB involved in kimchi fermentation belong to genera *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Pediococcus*, etc. (Park et al., 2012). Various studies have established that majority of the flora found in kimchi are dominated by *Leuconostoc* spp. and *Lactobacillus* spp.; however, minor colonies of microbial communities like *Pediococcus* spp. and *Streptococcus* spp. are also present (Lee, Ko, & Ha, 1992). Among the predominant species of LAB that have been isolated and successfully sequenced by suitable molecular techniques are *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Leuconostoc citreum*, *Leuconostoc gasicomitatum*, *Leuconostoc gelidum*, *Lactobacillus sakei*, and *Lactobacillus brevis*. A study conducted by Kim and Chun (2005) revealed the presence of different microbial communities like *Weissella koreensis*, contrary to this other groups of researchers, Lee et al. found a different set of species predominantly present in commercial kimchi, for example, *Weissella confusa*, *L. citreum*, *L. sakei*, and *Lactobacillus curvatus*. Due to a vast variation in raw material combination, incubation conditions, temperature, and pH of the medium of kimchi, the microbial medium represents an immense diversity in the microbial cultures and makes it highly complicated to control the growth or proliferation of a

single microbial community to produce a product of desired quality. However, the use of different microbial communities used in isolation by different researchers as starter cultures has resulted in a high-quality kimchi product with additional health benefits, for example, *L. citreum* (Chang, Shim, Cha, & Chee, 2010), *L. mesenteroides*, and mixed cultures of *L. mesenteroides* and *L. plantarum* (Bong, Jeong, & Park, 2013).

4.4 Health benefits of kimchi

The nutritional functionality of kimchi granted it the status of the world's healthiest food by health magazine. The health functionality attributed to kimchi is backed by the presence of various metabolites, microbiota, and biochemical compounds like vitamins (vitamin C, β-carotene, vitamin B complex, etc.), minerals (Na, Ca, K, Fe, P, etc.), chlorophyll, phytochemicals such as benzyl isothiocyanate, indole compounds, thiocyanate, and sitosterol (Park et al., 2012). Kimchi is also being credited as the richest source of various organic acids produced during the course of fermentation, amino acids, peptides, ornithine, and mannitol. The presence of these vast ranges of compounds having different biochemical functions exerts a well-demonstrated effect against various disorders and syndromes thereby contributing immensely to the betterment of human health. The nutraceutical properties attributed to kimchi include anticancer, antiobesity, antihypertensive as well as antimicrobial, and immunomodulatory properties. As kimchi preparation involves a mixture of a range of different raw materials that may vary from green vegetables to spices, the presence of different phytochemicals, essential oils, and pigments contributes to the total therapeutic properties of this fermented product (Park, Baek, Rhee, & Cheigh, 1995).

4.4.1 Anticancer properties

The anticancer properties of kimchi have been well established wherein various studies have positively shown the antimutagenic, anticlastogenic, and antiproliferative action of kimchi. A group of researchers (Park et al., 1995) successfully evaluated the antimutagenic properties of medium fermented kimchi against two potent toxins like aflatoxin B1 and (*N*-methyl-nitro-*N*-nitrosoguanidine). Mouse embryo cells C3H/10T1/2 that are mainly involved in the foci formation during the carcinogenic treatment in culture media developed very well as type II and type III and in turn allied well with tumor formation in almost 50% and 85% of the mice populations. Upon the treatment of the C3H/10T1/2 with methanolic extract fraction isolated from kimchi, a significant decrease in the transformation process was noticed. A study conducted by Ryu and Park showed that an exemplary antimutagenic effect was shown by kimchi extract in the drosophila test conducted in vivo and a significant anticlastogenic effect was also observed in mitomycin C–induced mice based on an in vivo supravital staining micronucleus assay. The antiproliferative action of kimchi as well as the cytotoxic effect of kimchi has been very well established against a range of different cancer cell lines. Different in vitro assays like MTT, sulforhodamine B, and growth inhibition assays specified the inhibitory effect of kimchi extracts on the growth of human AGS, gastric cancer, HT-29 colon cancer, MG-63 osteosarcoma, HL-60 leukemia, and Hep 3B liver cancer cells (Cho, 1999). Different biochemical metabolites like β-sitosterol and a linoleic acid formed during the fermentation process of kimchi have been shown to exhibit anticancer activity. The metabolites extracted by dichloromethane phase ought to arrest the G2/M phase of the cell cycle to destroy the HL-60 human leukemia cells. When transformed HIRC-B cells were treated with the dichloromethane fraction of the kimchi containing β-sitosterol as the active compound in combination of oncogenic H-rasv12, the growth rate of the cells was decreased owing to its blocking action on the DNA synthesis as well as modification of the signal transduction pathway (Cho, 1999). In a study conducted by Hur, Lee, and Lee (2004) sarcoma180 cells transplanted in Balb/c mice were treated with methanolic extracts of medium fermented kimchi and it was seen that the tumor weight was gradually decreased in the kimchi-treated samples compared to control. The antioxidative potential of the kimchi extract resulted in the reduction of the malondialdehyde formation in the culture medium as compared to the control samples. The methanolic extract was also shown to increase the enzymatic activity of major enzymes involved in detoxification system of live like glutathione *S*-transferase (GST) and glutathione reductase. In certain cases the activity of immune booster natural killer cells was also seen to increase upon treatment with kimchi extracts. Several studies have established the fact that kimchi acts as a potent anticancer and antiinflammatory agent due to its effect on a number of cancer-regulating pathways. In a study conducted by Kim et al., medium fermented kimchi was shown to successfully initiate the apoptosis of HT-29 human colon cancer cells by regulating the activity of Bax protein and mRNA involved in the macrophagic action of the colon cancer cells. In another study, Kim et al. revealed the anticancer and antiinflammatory action of kimchi extracts against colitis-associated colon cancer in mice induced by azoxymethane (AOM) and dextran sulfate sodium. The anticancer effect of kimchi was also observed in various sample groups wherein the kimchi-treated groups showed an increased colon length and lesser tumor growth. The active ingredients mainly involved in the anticancer properties of kimchi are baechu cabbage in combination with various other ingredients like mustard seeds and Chinese pepper. The anticancer effect of kimchi in

this case can be attributed to its ability to downregulate the protein expression of iNOS and COX-2 of the colon tissue of the mice.

4.4.2 Antioxidative effects

The antioxidative and antiaging effect of kimchi can be attributed to the presence of various pigments like chlorophyll, anthocyanins, carotenoids, phytochemicals like phenolic compounds, and other biochemical compounds like vitamin C, dietary fiber, and the microbial flora present. A study conducted by Hwang and Song (2000) revealed that the dichloromethane fraction of the kimchi exhibited antioxidative effect against lipid oxidation by quenching the Thiobarbituric acid reactive substances (TBARS) that are the secondary radicals involved in lipid peroxidation. Various studies pertaining to assess the antioxidative potential of kimchi have been carried out, out of which one study on human skin cells in which keratinocytes (A431 human epidermoid carcinoma) and fibroblasts (CCD-986SK human normal control cells) were cultured under oxidative stress full conditions induced by hydrogen peroxide. The effect of kimchi extract on both the normal cells and the control was assessed and the study revealed that optimally ripened kimchi successfully decreased the H_2O_2-induced cytotoxicity in the keratinocyte cells. Fermented kimchi from 1 to 3 weeks of storage also showed a strong antioxidative behavior against cell toxicity induced by paraquat at a concentration of more than 1 mM on keratinocyte cells, and simultaneously a reduced cell viability caused by induced H_2O_2 cell toxicity was also reversed by treating the cells with 2-week fermented kimchi extract effects. Stress-induced premature senescence (SIPS) model was used by Kim et al. to evaluate the antiaging effect of kimchi extracts on different human skin cells. In this model, human fibroblasts (WI-38) were treated with hydrogen peroxide that results in the reduction of cell viability with a simultaneous decrease in cell growth and increase in lipid oxidation thereby increasing the SIPS. Upon treatment with kimchi a successful increased cellular growth was seen and all the factors that led to lipid peroxidation were reversed thereby finding a possible way of using kimchi as a potential antiaging agent.

4.4.3 Hypolipidemic properties

A random trail to assess the hypolipidemic properties of kimchi was carried out by Kwon, Chun, Song, and Song (1999) on 106 Korean adult subjects with an age bar of 40–64 years. The Korean subjects were subjected to different biochemical analysis like blood examination, food records, and preferences for taste and personal life habits were assessed from time to time. The subjects after being fed by kimchi for given time intervals were again subjected for these analyses, and simultaneous increase in calcium levels and total dietary levels was observed. At the end of the study, it was concluded that a kimchi consumption of up to 500 g/day was correlated with an overall increase of high-density lipoprotein (HDL) cholesterol and simultaneous decrease in low-density lipoprotein (LDL) cholesterol. A study conducted by Lee, Song, Han, and Han (2012) concluded that consumption of kimchi led to a significant increase in HDL with a simultaneous decrease in the overall body mass and fat deposition. In addition to elevation of HDL cholesterol, a reduction in triglycerides was also seen. In a separate study conducted by Kim and Lee, different types of kimchi with varying fermentation periods (unfermented, 3 and 6 weeks) were fed to 63 male Sprague Dawley rats for 4 weeks at a proportion of 5% and 10% diet weight and in the due course of time a significant decrease in serum lipid levels and triacylglycerides was seen than the control group. It was further concluded that fermented kimchi was more effective in controlling the serum lipid levels and triglycerides than the subjects who were fed with nonfermented baechu kimchi, and fermentation process was directly responsible for increasing the functional biochemical components of the fermented kimchi that are in turn responsible for exerting the nutraceutical functionality on mammalian physiological systems. The study also confirmed the role of kimchi in extraction of triacylglycerols in feces to a much higher extent than the control group signifying the much higher fat mobilization and lipid extraction via feces indicating a lesser fat and lipoprotein absorption.

5 Tempeh

Tempeh is a combined term associated with all fermented beans especially soyabeans produced by living molds into a mushy framework (Nout & Kiers, 2005). Tempeh finds a unique place as a traditional, staple, and heritage food particularly in South East Asian countries especially in Indonesia and Malaysia for several years. Like most of the fermented foods across the globe, tempeh also has a long history of evolution that originated in Central Java (Indonesia). From there it spread to other parts of South Asian nations. The production pattern of tempeh varies across the world and is mainly dominated by family-owned small-scale producers. Tempeh and processed tempeh products like tempeh snacks are manufactured at cottage- or small-scale level and occur in a diffused manner making it cumbersome to determine the exact level of tempeh production. In Indonesia and Malaysia that are the niche regions of tempeh production, the small-scale production typically

varies from 30 to 50 kg of tempeh produced on daily basis. Tempeh has gained popularity in the world, especially in the diets of vegetarians and in developing countries, because it is easy to prepare and low in cost. Due to its nutty flavor and unique textural firmness, tempeh finds an important place as an important ingredient in preparation of sandwiches, soups, salads, etc. Production of tempeh is an easy and cost-effective technique for effective utilization of soybeans that cannot be consumed in raw form despite being a sink of proteins. In this case, fermentation is the most effective and efficient practice to harness the nutritional components of soyabeans by way of producing different fermented products. Tempeh offers the best ever substitute of meat because of unique composition as it contains more protein and less fat. It is because of this composition that tempeh started its career as poor man's meat. Nowadays, because of its affordability, nonmeat nature, health, and nutritional functionality, it is considered the most promising protein source among by the populace. Most of the vegans acknowledge tempeh as a quality diet due to its high protein content (40%–50% dry matter) and as a potent meat replacer, including fish. Based on preparation methods and the types of microbial strains used, fermented soyabeans with different consistency, flavor, aroma, and texture can be produced. Mostly, *Rhizopus oligosporus* the mold strains are used for the preparation of solid cake like tempeh. Tempeh is gaining attention in day-to-day market owing to its complete essential amino acid profile besides having a good quantity of isoflavones that impart various beneficial effects on human health (Khasanah, Ratnayani, Angwar, & Nuraeni, 2015; Soka, Suwanto, Sajuthi, & Rusmana, 2014).

5.1 Manufacture of tempeh

The traditional manufacturing process of tempeh typically utilizes the yellow soybean as a raw material; however, various other cereals like barley, peas, oats, chickpeas, lima beans, etc. can also be used as potent raw materials for tempeh preparation (Nout & Kiers, 2005). The process of tempeh manufacture can be categorized into various steps, that is, soaking, boiling of the beans followed by the addition of microbiota, which includes a mold starter preferably *Rhizopus* sp. molds especially *R. oligosporus*, *R. oryzae*, *R. arhizus*, *R. stolonifera*, and *R. microspores* and incubating at room temperature till the required consistency and flavor is reached. The process starts with dehulling of the beans followed by soaking of the beans for almost an hour or so till the moisture of the beans has increased to a certain content to support microbial growth. Soaking has some additional benefits that include, besides rendering the beans edible, helping in extraction of various antinutritional factors like saponins, tannins, etc. After soaking the beans, excess water is drained before by cooking. Afterward the beans are cooked for 20 minutes and spread in open for drying and then inoculated using starters of *Rhizopus* spp. and *Mucor* spp. and then subsequently packed and incubated for 48 hours at room temperature. Pure cultures of *R. oligosporus* are used in tempeh, and usually across different countries, no standard methods are followed—it usually depends on the taste of the consumers and marketability of the product (Nout & Kiers, 2005). Live cell count of the starters plays an important role in determining the quality of tempeh wherein lower levels of mold culture result in uneven mold growth that leads to higher fermentation times and gives rise to spoilage suggesting that the mold starter culture inoculated should be optimum preferably in the range of 102–106 CFU/g. In order to get an end product of optimum quality, the raw material mixed with the mold culture should be packed in anaerobic conditions flourishing the germination of the mold in an even manner. After the fermentation process is over, fresh tempeh is unpacked and first cooked, for example, in stews, or fried in oil to give delicious crisps (tempeh kripik), or in a variety of dishes as well as applied as an enrichment ingredient for formulated foods (Vaidehi, Sumangala, & Vijayakumari, 1996). Vast biochemical changes occur during the fermentation process of tempeh that mainly involves changes like release of bound minerals like iron, release of essential amino acids, etc. During the course of tempeh fermentation, the fungal strains facilitate the release of various enzymes like lipases, amylases, and proteases that in turn ease the hydrolysis of macronutrients releasing health beneficial phytochemicals, isoflavones, etc. (Ahmad, Ramasamy, Majeed, & Mani, 2015; Starzynska-Janiszewska, Stodolak, & Mickowska, 2014). Other biochemical changes that increase the bioavailability of major nutrients in tempeh include increase in the free amino acid profile of tempeh that is mainly caused by the action of proteases on the protein content of soyabeans (Murata, Ikehata, & Miyamoto, 1967; Murata, Miyamoto, Kokufu, & Sanke, 1970). Lipases produced by the fungal strains increase the free fatty acid content of tempeh that ultimately leads to a decrease of the lipid content of tempeh. With a major change in the organic macromolecules of tempeh, certain inorganic components like iron, calcium, etc. are also released from the dense network of macronutrients by hydrolyzing them. Various studies have shown an increase of folic acid profile of rats after being fed with tempeh over a definite period of time (Khasanah, 2013). Other important changes that occur during the course of fermentation of tempeh include the ones that increase the bioavailability of different minerals by the way of decreasing the activity and concentration of antinutritional compounds like phytates, saponins, and hemagglutinins, etc. It has been revealed by different studies that during tempeh fermentation, almost 70% decrease in the concentration of antinutritional compounds is observed due to the enzymatic action of the yeast colonies (Nout & Kiers, 2005).

5.2 Health benefits of tempeh

5.2.1 Antioxidant properties

Many degenerative disorders such as cancer, diabetes, atherosclerosis, arthritis, and neurodegenerative disorders occur due to the reactions caused by reactive oxygen species (ROS) (Shinde, Malpathak, & Fulzele, 2010). Various ROS give rise to free radicals that cause a cascade of different degenerative process that in turn leads to oxidative damage to the basic cell components like DNA, RNA proteins, and lipids. The majority of these free radicals are generated during the normal cellular metabolism; however, certain processes like oxidation and exposure to different mutating agents accelerate the production of these components on a higher scale. A chain reaction of these free radicals cripples the biological activity of the human system in addition of reducing the immune power (Droge, 2002). Tempeh, an isoflavone-rich product, has been shown to possess a high antioxidant capacity owing to its ability to quench singlet oxygen radical species. This property of tempeh is widely significant due to the high amount of isoflavone content (Lin, Wei, & Chou, 2006). Several studies conducted to verify the antioxidant potential of fermented soybean showed that the fermented soybean isoflavone extract (IC_{50} = 10 mg/mL) possessed a lesser DPPH free radical scavenging activity than isoflavones extract from tempeh (IC_{50} = 2.67 mg/mL); however, both of these extracts showed a similar chelating activity against FRAP (IC_{50} = 10.40 mg/mL, soybean and 11.13 mg/mL, tempeh) (Ahmad et al., 2015). Much of the antioxidant activity was shown to increase on the incubation of tempeh fermented with *Aspergillus niger* as compared to unfermented soybeans against peroxides and superoxides (Sheih, Fang, Wu, & Chen, 2014). In addition to this, an increase in the antioxidant activity against DPPH radicals was also seen on fermentation of tempeh with lactobacillus culture (Starzynska-Janiszewska et al., 2014).

5.2.2 Anticancer properties

With the production of fermented foods on a much higher scale, various researchers have focused their studies on elaborating the nutritional and nutraceutical role of fermented food products in elimination of various life-threatening diseases like cancer. With the support of numerous epidemiological studies, a direct relation has been found in the reduction of various cancers like hormone-dependent cancers, breast cancer, and prostate cancer due to the consumption of fermented soybean and associated products. The possible mechanism of action of soybean constituents to mitigate cancer is said to be the ability to modulate the cell cycle mostly at initiation and promotion stages to cripple the cancer progression of almost all cancer types, including breast cancer that is leading cause of mortality in women across the globe. Among the different constituents of soybean isoflavones, genistein and daidzein are said to exhibit the maximum chemopreventive activity against all the types of mutagenic disorders particularly against breast and prostate cancers (Taylor, Levy, Elliott, & Burnett, 2009). Genistein and daidzein are the major phytoestrogens present in soybean that are known to exhibit a weak estrogenic activity and are similar in action to human 17β-estradiol (Messina & Hilakivi-Clarke, 2009). A study conducted by Aggarwal and Shishodia (2006) and Li et al. (2008) revealed that genistein exhibits a unique mechanism by which it inhibits the activity of tyrosine kinases, topoisomerases, and other enzymes involved in DNA synthesis by its ability to curb the different cell signaling junctions involved in cancer cell growth. Xu, Xia, Hh, and Ck (2002) conducted a study in which he extracted the major biochemicals from the fermented tempeh like genistein, glycitein, daidzein, etc. On exposure of these biochemical to different cell lines like MCF-7 (breast cancer cell line), HeLa (immortal cervical cancer cell), and HO-8910 (ovarian cancer cell), he found that genistein was successfully able to inhibit the tumorigenic cell lines and daidzein had significantly reduced the progression of HO-8910 cell line. Overall, the activity of tempeh isoflavones was shown to be more significant in inhibiting the tumor growth rate at almost 31%; in addition to this, it was also able to increase the thymus index of macrophages in BALB/C mice impregnated with S-180 sarcoma cancer cells. Genistein, the major isoflavone present in tempeh, was shown to exhibit a strong tendency to decrease the mutagenic activity of 5-fluorouracil against pancreatic cancer (Suzuki et al., 2014), whereas Pavese, Krishna, and Bergan (2014) postulated that genistein has the ability to inhibit the cell detachment and decrease enzyme production in addition to cell invasion. Genistein in combination with daidzein had shown a strong cancer preventive action against isogenic human prostate cancer cells, such as LNCaP and C4-2B cells, when compared with individual soybean isoflavone (Dong, Xu, Sikes, & Wu, 2013).

5.2.3 Hypolipidemic properties

Among various life-threatening syndromes, CVDs are the leading noncommunicable diseases involved in human mortality across the globe. Nowadays, different dietary approaches are being taken to combat this disease, out of which protein intake is said to be the key player in decreasing the atherosclerotic risk both in animals and human. Tempeh, a protein-rich diet, has been shown to successfully decrease the concentration of triglycerides and LDL cholesterol in plasma (Carroll, 1991). A study based on human subjects revealed that tempeh proteins have the ability to reduce the total concentration of triglycerides

and total cholesterol in plasma and liver (Lin, Meijer, Vermeer, & Trautwein, 2004). Another study conducted by Gorcia Hermosilla et al. (2014) concluded that tempeh has the ability to reduce the enzymatic activity of hydroxymethylglutaryl-CoA (HMG-CoA) reductase that is widely responsible for the synthesis of cholesterol in mammalian liver. A human study conducted by Astuti et al. revealed that on consumption of tempeh-based instant formula by a group of 24 volunteers (8 males, 16 females) for a period of 3 months, a significant decrease was seen in the malondialdehyde and total cholesterol levels at the end of the feeding period. Malondialdehyde is the main decomposition product of fatty acids resulting in lipid peroxidation and a main damage causative agent in nucleic acids, proteins, etc. At the end of the study, it was concluded that the total cholesterol decreased by 8.6% and 10.25% in males and females whereas the LDL cholesterol levels decreased at a rate of 12% in male and 9.67% in female respondents, and then increased 9% in males and 15.5% in females after 2 months not consuming the tempeh. A significant decrease in uric acid levels (14%) was also seen in the female group on the consumption of tempeh for a trial period of 2 months.

5.2.4 Antineurodegenerative properties

Neurodegeneration is the process in which a series of nervous dysfunction occurs due to death of neurons, which ultimately results in age-related diseases like dementia and memory loss. The cognitive function of soybean isoflavone has become a matter of debate now due to its close resemblance with estrogen such that it has the ability to ameliorate neurodegenerative diseases in females (Duffy, Wiseman, & File, 2003). A study conducted by Ahmad, Ramasamy, Jaafar, Majeed, and Mani (2014) concluded that rats when fed with tempeh isoflavone extract for a period of 15 days resulted in a reduction of working memory error (WME) or improvement in the short-term memory. The reference memory error (RME) and WME are two variables that report the physiological status of the brain (Titus et al., 2007). A temporary remembrance of past events is called working memory or short-term memory, whereas long-term memory represents the well-learned responses in the present stimulus (Titus et al., 2007). The RME was significantly reduced that resulted in a well enhancement of long-term memory on the consumption of tempeh isoflavone extract (Ahmad et al., 2014). A number of different studies conducted on the elderly populations of Central Java revealed that a regular consumption of tempeh resulted in a significant improvement of immediate recall that is an ability to remember small bits of certain events over few seconds of time particularly in the younger population (Hogervorst et al., 2011). The study also concluded that elderly population was not receptive to the immediate recall on the consumption of tempeh at all.

6 Kombucha

Kombucha or Kombucha tea is a refreshing beverage produced by the fermentation of tea in combination with sugar by a multiculture microbial community mostly comprising yeast and bacteria. Kombucha tea is basically originated from China around 220 BCE and has been invariably sought to have magical properties by the Tsin Dynasty that ruled over China over many years. As the time evolved the practice of kombucha making and consuming spread across the European nations wherein extensive research was conducted on studying the nutritional and therapeutic properties of the beverage. In Russia, it most sought treatment to combat various ailments like metabolic diseases, hemorrhoids, and rheumatism. Production of this beverage varies from household consumption to market level, and from the last few years, Kombucha is sold across the globe with different flavors under different brand names according to consumer acceptance (Dufresne & Farnworth, 2000; Watawana, Jayawardena, Gunawardhana, & Waisundara, 2015). At the initial stage of fermentation the traditional Kombucha is mildly sour, has a vinegar-like taste close to apple cider but as the fermentation progresses, the taste of the beverage develops step by step from fruity to strong acidic taste adding to the total organoleptic properties of the beverage (Goh et al., 2012; Watawana et al., 2015). Kombucha can be prepared by following the traditional methods that involve boiling tea leaves mainly black tea leaves with sugar preferably sucrose under intense heat for 10 minutes or so after which more quantity of sucrose is added to the hot tea and fully dissolved under vibrant stirring and the preparation is left to cool down. After that, some acidified starter-like vinegar is added followed by addition of the starter fungus. The vessel containing the tea preparation is packed tightly to avoid any air contact and allowed to incubate for about 2 weeks at 30°C. After the incubation period is over, the vessel is opened gently. A layer of tea fungus is formed on the tea surface that is gradually removed from the tea surface; other fungal strips are removed by filtering the tea through a clean cloth after which the cold kombucha beverage can be stored in tight-capped glass bottles under refrigeration conditions. During this course of preparation, the taste and aroma of kombucha changes from a fruity flavor to a sour, vinegar-like taste as the storage period proceeds (Blanc, 1996a; Sievers, Lanini, Weber, Schuler-Schmid, & Teuber, 1995a). Different varieties of raw material can be used for kombucha preparation such of tea. The two main varieties of tea utilized for kombucha are *Camellia sinensis* var. sinensis and *C. sinensis* var. assamica (Hara, Hiroyuki, Nobuhide, & Shinji, 1995). The tea polyphenols contribute to the major nutraceutical potential of the kombucha beverage.

6.1 Probiotic potential of kombucha

The probiotic potential of kombucha has been extensively studied by various researchers (Dufresne & Farnworth, 2000). Different factors like change in geographical locations, microbial cultures, starter cultures, source of raw materials, and other environmental conditions affect the composition of kombucha. The symbiotic association of bacteria and yeasts provides powerful environment for the fermentation process of kombucha and also acts against the growth of contaminating microbes during this process (Balentine, Wiseman, & Bouwens, 1997). Amongst various bacterial cultures that are present in kombucha are acetic acid– and gluconic acid–producing bacteria such as *Acetobacter xylinum* that has been shown to be the primary bacterium present in the tea mat and a major producer of acetic acid and alcohol (Balentine et al., 1997) such as *Acanthodica xylinoides, Acetobacter aceti*, *Acetobacter pasteurianus* (Liu, Hsu, Lee, & Liao, 1996a), *Acetobacter suboxydans*, *Gluconobacter oxidans*, *Gluconobacte europaeus*, *Gluconobacter saccharivorans*. The majority of the tea fermentation process is carried out by tea fungus in association with bacterial cultures that are activated by the addition of disaccharides like sucrose (Cetojevic-Simin, Bogdanovic, Cvetkovic, & Velicanski, 2008; Battikh, Bakhrouf, & Ammarb, 2012a). There are a vast variety of yeast culture involved in kombucha tea such as *Saccharomyces cerevisiae*, *Saccharomyces ludwigii*, *Zygosaccharomyces bailii*, *Kloeckera apiculata*, *Schizosaccharomyces pombe*, *Brettanomyces bruxellensis*, *Brettano mycelambicus*, *Brettanomyces custersii*, *Torulaspora delbrueckii*, *Candida stellata*, *Candida stellimalicola*, *Candida tropicalis*, *Candida parapsilosis*, *Lachancea thermotolerans*, *Lachancea fermentati*, *Lachancea kluyveri*, *Torulopsis* spp., *Pichiamexicana*, *Eremothecium cymbalariae*, *Eremothecium ashbyii*, *Kluyveromyces marxianus*, *Debaryomyces hansenii*, *Meyerozyma caribbica*, *Meyerozyma guilliermondii*, *Hansenia sporauvarum*, *Hansenia sporameyeri*, *Hansenia spora vineae*, *Zygowilliopsis californica*, *Saccharomycopsis fibuligera*, *Merimblaingel heimense*, *Sporopachy dermialactativora*, *Kazachstania telluris*, *Kazachstania exigua*, *Starmera amethionina*, and *Starmera caribaea* (Teoh, Heard, & Cox, 2004; Malbasa, Loncar, Vitas, & Canadanovic-Brunet, 2011; Chakravorty et al., 2016; Coton et al., 2017). A group of researchers suggested that the majority of the LAB such as *Lactobacillus kefiranofaciens* subsp. *kefirgranum* become activated usually at the end of the fermentation process (Marsh, O'Sullivan, Hill, Ross, & Cotter, 2014). Other groups of researchers showed that the majority of the acetic acid bacteria like *L. plantarum*, *Streptococcus thermophilus*, *Streptococcus bovis*, *Streptococcus lutetiensis*, *Brevibacterium* (Petrušic, Radulovic, Paunovic, & Obradovic, 2011) form bulk of the tea microflora. The bacterial and yeast cultures are mainly involved in the production of various food acids like gluconic acid from the conversion of glucose and fructose as well as production of ethanol that also facilitates the growth of acetic acid bacteria that show antimicrobial action against various pathogens (Sievers, Lanini, Weber, Schuler-Schmid, & Teuber, 1995b; Liu, Hsu, Lee, & Liao, 1996b). The majority of the bacteria present in kombucha tea have also shown immunomodulatory effects (Marteau & Rambaud, 1993). The yeast–bacteria symbiotic association is the major precursor for the development of biocellulose type of mesh that triggers the growth of beneficial microorganisms inside the human gastrointestinal network. This beverage due to its immense microflora and nutraceutical potential provides an important diet for workers who work under challenging environments.

6.2 Biochemical profile of kombucha tea

The biochemical profile of kombucha tea reveals the presence of a vast group of different compounds both organic and inorganic in nature. As tea forms the major raw material in kombucha preparation, a vast group of different tea components form the bulk of the tea components (i.e., catechins, theaflavins, thearubigins, and flavonols). The predominant ones are catechins (flavan-3-ols): (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (−)-epigallocatechin gallate (EGCG), (+)-catechin (C), and (+)-gallocatechin (GC) (Hara et al., 1995). The astringent flavor and bitter taste of the beverage are contributed to the presence of these compounds. Kombucha consists of various disaccharides like fructose, sucrose, and monosaccharides like glucose are also present; in addition to them, certain organic acids like acetic, gluconic, glucuronic, usnic, lactic, citric, malic, succinic, pyruvic, tartaric, malonic, and oxalic acids derived from the carbohydrate substrates are also present in the fermented tea medium. Various studies have found a good quantity of vitamins preferably B group vitamins (B1–B12), folic acid, and vitamin C plus some minor components like aldehydes, ketones, esters, ethanol, fluorine, chlorine, bromine, iodine, phosphate, sodium, etc. (Jayabalan, Baskaran, Marimuthu, Swaminathan, & Yun, 2010; Velicanski, Cvetkovic, Markov, Tumbas Šaponjac, & Vulić, 2014). Ethyl-gluconate, oxalic acid, saccharin acid, keto-gluconic acids, succinic acid, and carbonic acids were other components typically found in the kombucha samples. Various pigments like chlorophyll, carotenoids, and various volatile compounds that are particularly involved in the aroma development of the brew are also present (Hara et al., 1995).

6.3 Health benefits of kombucha

6.3.1 Anticancer properties

A robust resistance to cancer has been found by various cancer research institutes upon the consumption of kombucha by different sets of rural populations across Russia (Dufresne & Farnworth, 2000). Various researchers have found a synergistic reducing mechanism of the different biochemical components of kombucha tea such as glucuronic acid, lactic and acetic acids, and antibiotic compounds on the proliferation of cancer vis-a-vis modulation of the cell-mediated immune system (Danielian, 2005). A strong correlation between the consumption of kombucha tea and reduction in cancer incidence has been reported by various researchers (Jayabalan et al., 2011, 2010) wherein they declared this capability of the kombucha tea occurs due to the presence of a variety of tea polyphenols and secondary metabolites produced during the course of fermentation. The study concluded that the possible mechanism of action by which the constituents prevent cancer proliferation might involve crippling of gene mutagenesis, modulating the cell cycle stages, inducing cell-mediated apoptosis of the cancer cells, and inhibiting angiogenesis of cancer cells. It has also been seen that kombucha tea has the ability to decrease the tumor cell resistance by reducing the cell proliferation with a simultaneous decrease in metastasis and reducing the cellular blood supply. A study conducted by Bhattacharya, Gachhui, and Sil (2013) revealed that upon induction of a dose of 150-mg kombucha extract per kg, body weight for a trail period of 14 days could enhance DNA damage of the pancreatic tissue of alloxan-induced diabetic rats. A study conducted by on rats by Ragy (2015) revealed that when mutagenesis was induced in rats by the induction of chromates that are well-known carcinogenic compounds, a significant increase in lipid peroxidation as well as oxidative stress was seen. When the rats were fed with kombucha tea beverage, a simultaneous decrease in the oxidative stress and associated changes were seen. Another study by Gharib (2014) to evaluate the effect of kombucha on reversing the carcinogenic effects caused by electromagnetic field was conducted. In this study the rat subjects were exposed to an electromagnetic field with a strength of field (950 MHz) for 8 weeks. After several physiological examinations, it was noticed that the electromagnetic field was sufficiently able to cause of series of carcinogenic processes like increasing oxidative stress and generation of free radical species. However, upon induction of kombucha tea, a substantial reversal of these adverse changes was seen indicating the antioxidant or antiradical properties of the kombucha tea on the mammalian physiological systems.

6.3.2 Antioxidant properties

A myriad health benefits have been accredited to kombucha tea owing to its unique biochemical profile. Different health benefits like protection from cancer induction, lipid-lowering effects, immunomodulatory properties, etc. are being attributed to kombucha by different researchers. A study conducted by Jayabalan et al. (2008) concluded that there occurs a significant increase in total phenolic content and antiradical activities of the kombucha tea during the course of fermentation, whereas other researchers (Malbasa et al., 2011) were of the opinion that the microbial cultures involved in the fermentation process extensively determine the antioxidant potential of the kombucha tea like the presence of *Zygosaccharomyces* sp. that increased the antioxidant capacity of the beverage many times. Bhattacharya, Manna, Gachhui, and Sil (2011) studied the antioxidant effect of kombucha tea against hydroperoxide-induced hepatocyte cytotoxicity wherein a significant decline in the oxidative stress as well as an increase in the cellular apoptosis was seen upon the induction of kombucha tea. It was also concluded that a positive correlation was seen between the antioxidant potential and the total phenolic content plus ascorbic acid of the kombucha tea. Yang et al. (2009) assumed that the antioxidant potential of kombucha tea was directly proportional to the microbial flora present in the beverage medium in addition to the biochemical profile and the fermentation period, but it was also seen that the content of phenolic substances in the medium enhanced the hydroxyl and superoxide radical scavenging capacity of the beverage. The presence of tea polyphenols in kombucha tea increases the antiradical activity of the beverage 100-fold than ascorbic acid and as many as 25-fold as compared to vitamin E.

6.3.3 Antimicrobial properties

The antimicrobial property or the pathogen impeding power of kombucha tea has been well documented by several studies. Sreeramulu, Zhu, and Knol (2000), Sreeramulu et al. (2000), Battikh, Bakhrouf, and Ammar (2012b) studied the antimicrobial properties of kombucha tea against a variety of pathogens like *Enterobacter cloacae*, *Aeromonas hydrophila*, *Agrobacterium tumefaciens*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Shigella sonnei*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Campylobacter jejuni*, *Helicobacter pylori*, and *Candida albicans*. The biochemical profile that includes bacteriocins, phenolics, organic acids present in the beverage are main components involved in pathogen inhibition; in addition to it, ascorbic acids have been seen to be the most potent antimicrobial agent present in the beverage medium (Yang et al., 2009), bacteriocins and

tea-derived phenolics may also be involved (Sreeramulu, Zhu, & Knol, 2001). In addition to antimicrobial activity, antifungal activity of the beverage has also been well established wherein a sufficient activity was seen against candida species. Sreeramulu et al. (2000) in his study concluded that most of the antimicrobial activity of kombucha was due to the presence of acetic acid plus some minor amounts of bioactive amines and catechins present in the beverage medium that showed much of the inhibitory activity against both Gram (+) and Gram (−) bacteria. The type of raw material used in the preparation of kombucha was also shown to have effect on the antimicrobial activity of the kombucha tea. A study conducted by Battikh et al. (2012a) revealed that kombucha when prepared from leaves of *Thymus vulgaris* L., *Lippia citriodora*, *Rosmarinus officinalis*, *Foeniculum vulgare*, and *Mentha piperita* showed an increased antimicrobial activity as compared to kombucha prepared from black tea only. The microbial cultures used in the fermentation process were also shown to exhibit strong antimicrobial properties. The incubation of kombucha with *L. citriodora* and *F. vulgare* resulted in a strong antimicrobial action against majority of the fungal species, for example, *Candida glabrata*, *C. tropicalis*, *Candida sake*, *Candida dubliniensis*, and *C. albicans*. The addition of cactus pear juice to kombucha raw materials during the fermentation process resulted in a strong inhibitory activity against *S. aureus*, *B. cereus*, *S. epidermidis*, and *Enterococcus faecalis*, *E. coli*, *Klebsiella pneumoniae*, and *P. aeruginosa* in contrast to the unfermented raw material extracts (Ayed & Hamdi, 2015).

7 Kefir

Kefir derived from Turkish word *Keyif* that indicates good feeling, pleasure, or well-being is one of the significant fermented probiotic dairy products, which is also considered to be an effective supplement. In recent times, it is also denoted by various other names like kefyr, kephir, kefer, kiaphur, knapon, kepi, and kippi. It is usually whitish or slightly yellowish in color and the taste varies from mild acidic to sour depending upon the fermentation conditions and extent of carbonation. Traditionally, Kefir is produced by lactic acid fermentation of milk by the addition of kefir grains, which contains a myriad symbiotic microflora that extensively ferments the carbohydrate content of milk derived from different species like cow, buffalo, sheep, camel milk, etc. by inoculation of kefir particles (Sharifi, Moridnia, & Mortazavi, 2017). Both at household and commercial levels of kefir production, the use of kefir grains as a starter culture has minimized to a certain extent wherein the use of the specific microbiota isolated from kefir grains is used to provide unswerving vehicles (Assadi, Pourahmad, & Moazami, 2000). During the fermentation process a number of compounds with unique composition are produced that contribute to distinct flavor and aroma to the end product that is contributed by a significant amount of alcohol, diacetyl, acetaldehyde, and acetoin and other flavor compounds that are produced during the fermentation process (Farnworth, 2005; Walsh et al., 2016). In addition to this, an exclusive X polysaccharide, kefiran, is also produced that wholesomely contributes to the textural, rheological, and organoleptic properties of the end product (Frengova, Simova, Beshkova, & Simov, 2002; Rimada & Abraham, 2006). By weight, kefiran comprises approximately 1/4th of kefir particles whereas kefiran itself consists of an amazing composition that includes approximately 30%–34% casein, 45%–60% saccharide, 3%–4% fat, a minor percentage of microbial load plus some disaccharides (Karatepe, Yalçın, & Patır, 2012). The production of kefir dates back to several centuries at around 100 BCE with various scriptures depicting its preparation and consumption in Central Asia (Arslan, 2015; Liger-Belair, 2016) and around 1900 BCE in Xinjiang, China. Until recently, kefir has found its place as an important drink in the European Union and is also widely consumed by different Asian countries across the globe (Loretan, Mostert, & Viljoen, 2003). Various other researchers argue the fact that consumption and origin of kefir as well as kefir grains took place in Caucasus Mountains of the Soviet Union (Libudzisz & Piatkiewicz, 1990). The traditional preparation protocol passed down from generation to generation, and generally, the possession of this knowledge determined the wealth of the people at those times.

7.1 Probiotic value of kefir

The microbiota of kefir grains, the key component in the production of kefir, comprise a number of different species of multiculture microorganisms, the most common ones being from *Lactobacillus* like *L. kefiri*, *L. kefiranofaciens*, *L. kefirgranum*, and *L. parakefiri*; characteristic yeasts in kefir are *Saccharomyces* spp. and *K. marxianus* (Kök-Taş, Ekinci, & Guzel-Seydim, 2012). The bacterial population mostly found in kefir comprises *Lactobacillus*, *Lactococcus*, *Streptococcus*, and *Leuconostoc* (Simova et al., 2002; Chen, Yanagida, & Hsu, 2006). The most dominant bacterial species that are present in both kefir grains and milk to be fermented with the starter culture are *Lactococcus lactis* subsp. *lactis*, *S. thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus casei* subsp. *pseudoplantarum*, *L. kefiri*, *Lactobacillus kefir*, and *L. brevis.* The nonlactic acid bacterial communities that have been successfully isolated from kefir comprise various genera like *Acetobacter* (Witthuhn, Schoeman, & Britz, 2005) and *Bifidobacterium* (Marsh, O'Sullivan, Hill, Ross, & Cotter, 2013). Among all the bacterial cultures, homofermentative *Lactobacillus* constitutes the

most important part of the bacterial flora out of which *L. kefiranofaciens*, performs an important role in overall fermentation process as it helps in the clubbing and formation of kefir grains (Karatepe, Yalçın, & Patır, 2012). Diacetyl and acetaldehyde, which are the major flavoring components, are produced by *Streptococcus lactis* subsp. diacetyl *lactis* and *Leuconostoc* sp. (Libudzisz & Piatkiewicz, 1990). Despite the variable bacterial population comprising bulk of the microbial load of kefir, some kefir grains are dominated by yeast species (Simova et al., 2002; Da Cruz Pedrozo Miguel et al., 2010). Among different yeast species, most commonly found and successively isolated from kefir grains and fermented milk are *Saccharomyces*, *Kluyveromyces*, and *Candida* (Angulo, Lopez, & Lema, 1993). Out of total microbial load of kefir, *Lactobacillus* comprise approximately 80% of the total microbial load the rest being occupied by *Streptococcus* (20%) and yeasts (5%) (Karatepe, Yalçın, & Patır, 2012). Different parameters that include type of preparation protocol used, source of milk, and geographical differences determine the overall end characteristics like quality, safety, organoleptic, biochemical properties, and the total microflora of kefir that ultimately vary the physicochemical and nutritional profile of the end product (Karabıyıklı & Das¸tan, 2016). Various studies have shown that the chemical composition and most of the known nutritional properties vary significantly with change in source of base materials like milk, starter culture viability, and the fermentation conditions (Zubillaga, Weill, & Postaire, 2001). Chemically kefir constitutes water as the major component followed by carbohydrates like kefiran and disaccharides as well as proteins and fat (Ozer & Robinson, 1999). Other minor components like CO_2, alcohol, and some fatty acids like butyric acid, lactic acid, acetic acid, pyruvic acid, hippuric acid, propionic acid, aroma compounds like diacetyl and acetaldehyde that are also adding some taste and aroma in the product are produced during the process of fermentation.

7.2 Kefir production

With the evolution of mankind and technology, different methods of kefir production have been developed but traditional methods with minor modifications are still in place. To produce end product of good quality and excellent consumer acceptance, various traditional process parameters are generally preferred. Basic raw materials like milk from any species like goat, sheep, buffalo, and cow, etc. are used for kefir production (Otles & Cagindi, 2003). Generally, the first and foremost step involves the preliminary examinations of basic raw materials like milk that includes microbiological examinations to determine the quality of milk which is homogenized and pasteurized to decontaminate it. Traditionally, kefir production involves addition of active starter culture mainly in the form of kefir particles directly to milk. Kefir is prepared batchwise wherein batches of milk are mixed with kefir particles (5%) usually at room temperature. The mixture is later on left for fermentation for 24 hours or so. After the fermentation is over, some of the kefir grains are separated out for next inoculation (Otles & Cagindi, 2003). At the end of the fermentation process, the kefir is bottled tightly and stored under refrigerated conditions for further use. Around various countries across the globe, different protocols are used for preparation and selection of the raw materials required for the production of kefir. Various postprocessing techniques are nowadays used for the efficient storage of kefir to minimize losses due to contamination. Refrigeration, freeze-drying, and freezing can be some of methods for storage of kefir and kefir grains that can keep the cultures viable for extended time periods. Among these, lyophilization appears to be the most promising approach for storage as it can keep the cells viable for almost up to 8 months (Turan & Ilter, 2007).

7.3 Health benefits of kefir

7.3.1 Anticancer properties

Generally, the consumption of fermented milk products has been seen to decrease the risk of the incidence of cancer especially breast cancer that is a leading cause of death in women across the globe (Reddy, Rag, & Reddy, 1987). Different studies have established this fact that the anticarcinogenic activity of fermented milk may be attributed to the biochemical profile of the fermented milk, which includes bioactive peptides, organic acids, and small amounts of vitamins and minerals. A study conducted by Svensson et al. (1999) confirmed the antimutagenic capacity of kefir against various cultured cancer cells in vivo. A possible mechanism of cancer prevention by kefir involved the modulation of cell cycle mechanism wherein the cell growth is seized at the initiation phase as well as by hindering the enzyme activity involved in cancer cell plus immune system activation. Impressing results were concluded by various studies against carcinogenic effects wherein kefir was found to suppress the metastasis process. Sharifi, Moridnia, & Mortazavi, 2017 stated that kefir extract had an extensive capacity to decrease the concentration of ROS in the intracellular systems with a significant increase in cellular apoptosis. Kefir extract was also seen to selectively target cancer cells without causing any physiological effect on normal cells (Jalali, Sharifi, & Salehi, 2016). Various studies also evaluated the anticarcinogenic effects of kefir on different human tissues. A study conducted by Grishina, Kulikova, & Alieva, 2011 evaluated the effect of kefir on human

colon wherein he observed that a significant decrease in DNA damage caused by different carcinogenic agents occurred that itself decreased the chances of carcinogenesis. Apart from biochemical composition of kefir, a significant volume of literature suggests a possible role of the microbial communities in deciphering the antimutagenic properties against various cancers. A study conducted by Miyamoto et al. (1991) revealed that isolated strains of *Lactobacillus*, *Streptococcus*, and *Leuconostoc* could bind mutagens to inactivate them. Kefir prepared from soymilk showed almost a twofold increase in anticancer activity due to the presence of isoflavones like genistein and daidzein (Liu, Chen, & Lin, 2002). Other researchers have given a good quantum of focus on the anticancer potential of kefiran, a water-soluble polysaccharide that is the main ingredient of kefir (Wang et al., 2008) in which the water-soluble portion of the polysaccharide matrix was shown to have an abundant antitumorigenic activity (Furukawa, Watanabe, Toyama, & Morinaga, 2013). A study conducted by Can, Topuz, and Derin (2009) suggested that the consumption of kefir beverage during the course of chemotherapy process was not sufficiently able to neutralize the chemotherapy side effects like gastrointestinal disturbances, nausea, vomiting, and mouth ulcer formation; however, it was able to relieve the sleeping disturbances in the patients undergoing the therapy.

7.3.2 Antiobesity properties

Weight gain is mainly associated with an alteration in the intestinal microflora in addition to intake of highly processed food components. The major success has been attributed to modulation of intestinal microbial flora to combat weight gain (Kim et al., 2015). A study conducted by Dong-Hyeon, Hyunsook, & Dana, 2017 revealed that obese rats when administered kefir for 12 weeks showed a drastic decrease in body weight than the control group that were administered milk for the same trail period. The intestinal microflora of the group consuming kefir showed an increase in the colonization of yeast and *Lactobacillus*/*Lactococcus* that were responsible for enhancing the fatty acid oxidation that in turn lead to the decrease in adipose tissue development and a simultaneous decrease in body weight. One more study conducted by Choi, Kang, & Lim, 2017 revealed that obese rats when fed with kefir significantly decrease the adiposis in live tissues thereby causing a steady decrease in fat accumulation in liver and epididymal tissues by decreasing the fatty acid synthesis. Yet another study conducted by Fathi, Faghih, & Zibaeenezhad, 2016 showed that the positive effect of kefir on a group of premenopausal women in the age group of 25–45 years divided into three groups for a trail period of 8 weeks. In this study, one group was administered with low-fat milk (two portions daily), and the second group was administered with low-fat milk (four portions daily), while the third group was administered with low-fat milk in combination with kefir (two portions each daily). All the parameters were checked on routine basis that included body weight, BMI, and waist circumference and it was found that the group who consumed low-fat milk in combination with kefir showed a significantly lower body weight than the other two groups preferably in the 8th week of the study.

7.3.3 Hypocholesterolemic properties

CVDs are one of the major causes of mortality among the global population in which a high blood cholesterol level plays a major risk factor in the development of atherosclerosis. Studies have suggested a major role of diet in combatting serum cholesterol levels wherein fermented milk products share a major part in decreasing the serum cholesterol levels (Bourrie, Willing, & Cotter, 2016). A study conducted by Rosa et al. (2017) elucidated the fact that microbial flora present in kefir has the capability to modulate cholesterol production by increasing the synthesis of a short-chain fatty acid the propionate HMG-CoA. Kefir boosts propionate production that in turn has the ability to inhibit gene expression involved in cholesterol biosynthesis thereby decreasing the serum cholesterol level. The microbial flora especially *L. plantarum* was able to sufficiently decrease the total cholesterol level in rats with high serum cholesterol levels (Wang, Xu, & Xi, 2009). Alp and Ertürkmen (2017) conducted several studies to determine the possible mechanism by which kefir is able to decrease cholesterol synthesis in mammals. It was seen that microbial floras of kefir particularly the *Lactobacillus* species are involved in production of bile salt hydrolase enzymes, which is mainly involved in the deconjugation of bile salt that successively decreases the bile acid solubility. As the solubility of bile acids get decreased after deconjugation, majority of it gets discharged from the intestines as such that imparts a positive feedback on liver to synthesize more and more of the bile acid. As such the majority of the cholesterol is utilized by liver to synthesize more and more of bile acids, which in turn decreases the total serum cholesterol.

7.3.4 Immunomodulatory properties

The possible effect of kefir on immune system has been shown to be due to its unique composition. Various studies have shown the presence of complex lipids, including sphingomyelin in kefir medium. Despite having no direct relationship between nutrition and immune response, a number of studies support the fact that the diversity in microbial communities

and their metabolites manipulate the activity of macrophages involved in pathogenic activity that ultimately affects the response system of the body. (Ahmed, Wang, & Ahmad, 2013) explained in his study that the microbial culture of kefir had a profound effect on the innate immune system of the human body wherein the immune responses of the body were modified by fluctuating the cytokine system of the body. (Adiloğlu, Gönülateş, & İşler, 2013) conducted a study to explain the possible effect of kefir on the immune system. In this study, healthy volunteers in the age group of 20–40 years were put on diet comprising nonfermented food products up to a period of 14 days and then afterward 200 mL of kefir beverage was included in the diet for a period of 6 weeks. A simultaneous decrease in the serum interleukin-8 levels in weeks 3 and 6 was observed, whereas interleukin-5 increased in week 3. With the increase in the time period of kefir dosage, a significant increase in the tumor necrosis factor (TNF-α) was observed that simultaneously leads to a decrease in the allergic response. The possible disease eliminating effect of kefir has also been studied by various researchers wherein it was shown that kefir particles showed an immense inhibitory effect against Giardiasis, caused by Giardia intestinalis thereby decreasing infection prognosis and strengthening the humoral and cellular immunity (Franco, Golowczyc, & De Antoni, 2013).

8 Sauerkraut

Fermented vegetables have a long history and have been used since antiquity, with sauerkraut being one of the most famous fermented products. Sauerkraut, a sour cabbage, is formed from the lactic acid fermentation of chopped and salted white cabbage (*Brassica oleracea*). The antecedents of fermented cabbage differed significantly from that prepared at present. Initially, the leaves of cabbage were dressed with vinegar or sour wine. Later, the cabbage was chopped and then packed into containers covered with verjuice (immature apple or grape juice), vinegar, or sour wine. Finally, the acids were replaced by salts and spontaneous fermentation was started (Raak, Ostermann, Boehm, & Molsberger, 2014a).

Sauerkraut is a German word for the term "sour cabbage." It has been defined by the FDA's Food Inspection Decision 196 as "the product obtained by the complete fermentation, mainly lactic, of properly prepared and shredded cabbage in the presence of 2% to 3% salt." It should contain not less than 1.5% lactic acid after the end of fermentation. The sauerkraut was first prepared 2000 years ago in China where rice wine was used to ferment cabbage. It was later introduced in Europe after 1000 years by Genghis Khan after invading China. Europeans substituted wine with salt in sauerkraut (Wacher, Díaz-Ruiz, & Tamang, 2010). Sauerkraut was originally made only in the home as it was a means of utilizing fresh cabbage that otherwise would spoil before its use. Now, the production of sauerkraut has started at the commercial level and has become an important food industry. However, a significant amount is produced in the rural and suburban areas where home vegetable gardens still exist. Cabbage varieties best suited for the growth in the major production areas are used early, midseason, and late types are grown. Varieties that were formerly used like Early Jersey Wakefield, Late Flat Dutch, Early Flat Dutch, and others have been replaced by new varieties that have been bred to be well-adapted to mechanical harvesting and inherently contain less water, thereby reducing the generation of in-plant liquid wastes. Mild-flavored, sweet, solid, white-headed cabbage is the best choice as it makes a superior kraut.

8.1 Production of sauerkraut

For sauerkraut manufacture, matured fresh cabbage heads are trimmed to remove the outer green broken or dirty layers. The cabbage cores are then shredded to 0.7–2 mm thick strips. Generally, long, finely cut shreds are preferred and the thickness is determined by the judgment of the manufacturer. The shredded cabbage, also known as slaw, is then moved to vats by the conveying belts for salting and fermentation. Salts play an important role during sauerkraut manufacture and the concentrations used should be carefully controlled. It is important for the development of anaerobic conditions during fermentation and for controlling the growth of undesirable microorganisms and activity of endogenous pectinolytic enzymes that cause cabbage softening. Salt also enhances the flavor of finished sauerkraut by maintaining a proper salt–acid balance if the cabbage is salted properly. The amount of sodium chloride added usually depends on fermentation temperature and the market preference (Wolkers-Rooijackers, Thomas, & Nout, 2013). According to legal standards authority, the concentration of the salt should be in between 2% and 3%. Therefore most of the sauerkraut producers use a concentration in the range of 0.7%–2.5% (Holzapfel). However, as per the new guidelines on salt intake published by WHO in 2012, there is a trend toward the reduction of salt concentration during sauerkraut manufacture. Sometimes, herbs, spices, carrot, and wine are added during fermentation to enhance the flavor of sauerkraut. The salted cabbage is then placed in fermentation vessels and pressed tightly to remove the air. These vessels are then hermetically sealed so that anaerobic fermentation of the cabbage occurs. The fermentation time may vary from 1 week to several months. After fermentation the sauerkraut is either consumed fresh or packed in metal cans or glass jars and pasteurized to increase its shelf life. Although sauerkraut is produced by spontaneous fermentation, its quality and flavor mainly depend on the type of indigenous microorganisms

and the quality of cabbage used for fermentation. Therefore change in the native microbiota of raw cabbage will influence the quality of sauerkraut (Lindow & Brandl, 2003).

In recent years, the use of starter cultures for sauerkraut manufacture has been proposed in order to reduce the variation in the quality and to ensure the production of uniform product. Presently, sufficient starter cultures suitable for cabbage fermentation are not available. Therefore the production of starter cultures for preparation of sauerkraut with desirable organoleptic properties is still a scientific challenge. The bacterial strain used as starter should be carefully selected in order to produce sauerkraut with optimum quality. The selection criteria should be based on the ability of the strain to adapt to cabbage material and tolerate to low pH and high salt concentrations. A rapid growth rate and the production of acids resulting in fast acidification of sauerkraut are also considered important criteria for the selection of starter culture. Moreover, a heterofermentative metabolism and synthesis of antimicrobial agents, including bacteriocins and hydrogen peroxide, are considered important features of starter cultures used for sauerkraut production (Di Cagno, Coda, De Angelis, & Gobbetti, 2013; Lalitha, 2016). Starter cultures with single or multiple strains may be used. Some studies have reported the use of starter cultures obtained from previous fermentation process (Beganović et al., 2014; Wiander & Ryhänen, 2005a). Recently, the use of different starter cultures was investigated to produce good-quality sauerkraut with increased content of bioactive compounds and reduced levels of salt and biogenic amines. A strain of Leuconostoc mesenteriodes (nisin-resistant) either alone or in combination with *L. lactis* strain (nisin-producing) has been used to inhibit the growth of naturally occurring LAB, thereby improving the uniformity of sauerkraut quality (Breidt, Crowley, & Fleming, 1995). The production of good-quality sauerkraut with substantial amount of phytochemicals has been prepared by Tolonen et al. (2002) by using a starter culture containing mixture of mesenteroides and *Pediococcus dextrinicus*. The same authors reported in another study that the introduction of *L. sakei* culture to sauerkraut promoted its antimicrobial activity and concentration of bioactive compounds two to three times higher than that produced by using other starter cultures (Tolonen et al., 2004). Furthermore, *L. mesenteroides* as starter culture in combination with mineral salt with low NaCl was used that produced a sauerkraut juice with mild taste, uniform sensory, and microbiological quality (Wiander & Ryhänen, 2005b). Johanningsmeier, McFeeters, Fleming, and Thompson (2007) in their study found that the addition of *L. mesenteroides* during sauerkraut preparation produced a more uniform product as compared to noninoculated fermentations while allowing 50% NaCl reduction. In another study conducted by Peñas, Frias, Sidro, and Vidal-Valverde (2010a), *L. mesenteroides* as starter culture with low salt concentration resulted in a significant retention of vitamin C and produced substantial amounts of ascorbigen (a glucosinolate hydrolysis compound with recognized anticarcinogenic properties) and reduced content of biogenic amines when compared to cabbages inoculated with *L. plantarum*, which was reported by Peñas, Frias, Sidro, and Vidal-Valverde (2010b). In another study a decrease in biogenic amines has been reported after addition of *L. plantarum*, *L. casei* subspecies casei 2763, or *L. curvatus* 2771 and 2775 as starter cultures in comparison to spontaneous fermentation (Kalač, Špička, Křížek, & Pelikánová, 2000; Rabie, Siliha, el-Saidy, el-Badawy, & Malcata, 2011).

8.2 Nutritional profile of sauerkraut

Cabbage is considered as a health-promoting food due to its high nutritional profile with significant amount of bioactive compounds. The major nutrients present in white cabbage are carbohydrates (4.18–5.51 g/100 g) and dietary fiber (1.9–2.9 g/100 g) followed by proteins (1.27–1.37 g/100 g), minerals (0.3–0.7 g/100 g), fat (0.06–0.20 g/100 g), and vitamins, particularly vitamin C (0.03–0.04 g/100 g). In addition, cabbage is also a rich source of phytochemicals, mainly phenolics and glucosinolates. Glucosinolates of the Brassica family have been extensively studied and have been found to have protective role against cancer. Its content in the cabbage depends on the environmental conditions, genotype, geographical growing location, and postharvest processing conditions (Peñas, Frias, Martínez-Villaluenga, & Vidal-Valverde, 2011; Verkerk et al., 2009).

The chemical composition of cabbage changes during fermentation, resulting in the increase in the contents of carbohydrates, proteins, fat, dietary fiber, minerals, and vitamins. Sauerkraut also contains organic acids mainly 1%–2% lactic and acetic acids with small quantities of propionic, malic, and succinic acids as well as ethanol, ethyl acetate, acetaldehyde, and carbon dioxide (Frias, Martinez-Villaluenga, & Peñas, 2016). It is regarded a good source of antioxidants that include vitamin C (14.7–75 mg/100 mg, fresh wt.) and phenolic compounds (0.44–1.06 mg gallic acid equivalents/100 g fw) (Peñas et al., 2011; Podsedek, 2007).

8.3 Health benefits of sauerkraut

The health-promoting effects of sauerkraut are well known since ancient times. Hippocrates, a Greek physician, recommended consumption of sauerkraut to reduce as a remedy against overweight, while Romans consumed it to reduce intestinal infections (Podsedek, 2007). Several research investigations and clinical studies have reported that consumption of Brassica vegetables is associated with reduced risk of chronic diseases, including cancer and CVDs (Hayes, Kelleher, & Eggleston, 2008; Tse & Eslick, 2014). The health benefits of sauerkraut are given in the following subsections.

8.3.1 Antioxidant properties

Oxidative stress occurs when the production of ROS is greater than the antioxidant activity of an organism. It has been found responsible for pathogenesis of many chronic diseases, including neurodegenerative diseases, CVDs, and cancer (Pisoschi & Pop, 2015; Persson, Popescu Bogdan, & Cedazo-Minguez, 2014). Sauerkraut being a rich source of vitamins, especially vitamin C and E as well as phenolic compounds, acts as a potential free radical scavenger, thereby providing protection against oxidative stress (Podsedek, 2007; Raak, Ostermann, Boehm, & Molsberger, 2014b). It has been found that vitamin C decreases the levels of C-reactive protein, responsible for inflammation and atherothrombosis and acts an electron donor for human enzymes, thus neutralizing superoxide and hydroxyl radicals (Ellulu, Rahmat, Patimah, Khaza'Ai, & Abed, 2015). Vitamin E shows its antioxidant activity due to hydrogen donating ability, providing protection against CVDs by inhibition of oxidation of LDL (Podsedek, 2007). The free radical scavenging activity of sauerkraut obtained under different fermentation conditions has been extensively investigated. The reported values of peroxyl radical scavenging activity was between 132 and 164 μmol Trolox/g (dry wt.) by using ORAC-FL in sauerkraut produced by natural or induced fermentation (Martinez-Villaluenga et al., 2012; Peñas et al., 2012). The antioxidant activity observed for sauerkraut in all studies was higher than that observed in raw cabbage.

8.3.2 Anticarcinogenic activity

Recently, some studies have reported that sauerkraut shows anticancer activity. Its chemopreventive activity has been attributed to the induction of phase II detoxifying and antioxidant enzymes by ITCs and other glucosinolate breakdown products like I3C and ABG produced during sauerkraut fermentation. A study documented that rats (1.25 mL/kg body weight) fed with sauerkraut juice for a period of 28 days showed increased expression and GST, NAD(P)H quinone oxidoreductase 1 (NQO1) activities (Krajka-Kuźniak, Szaefer, Bartoszek, & Baer-Dubowska, 2011), which are the two key detoxifying enzymes in liver. Many other studies have reported that role of sauerkraut in cancer prevention (Szaefer, Krajka-Kuźniak, Bartoszek, & Baer-Dubowska, 2012).

8.3.3 Ability to protect oxidative DNA damage

It is well established that increase in the levels of ROS can induce oxidative DNA damage resulting in DNA mutation that is a crucial step in carcinogenesis. Scott et al. (2014) documented the role of elevated levels of oxidized base lesions in DNA during the initiation, promotion, and progression stages of carcinogenesis. In a study conducted by Kusznierewicz et al. (2010), the sauerkraut juice was found to prevent DNA damage in human colon tumor HT29 cells that were exposed to an oxidative insult (300-μM H_2O_2). This could be due to the presence of isothiocyanates and indoles in sauerkraut as these phytochemicals have been found to provide protection against chemicals like benzo(*a*)pyrene and hydrogen peroxide that damage DNA in LS-174 colon cells. Other studies have also reported that isothiocyanates such as sulforaphane can protect DNA from chemicals in human hepatoma, mammary epithelial, and liver epithelial cells (Jiang, Chen, Yang, Hebbar, & Kong, 2003; Kusznierewicz et al., 2010).

8.3.4 Antiinflammatory effects

Inflammation has a crucial role in various chronic diseases, including cancer, T2D, CVDs, and obesity (Donath, 2014; Fernandes et al., 2015; Ueland et al., 2015). The endotoxins like Lipopolysaccharides (LPS) stimulate the process of inflammation and macrophages are considered the most important cells involved in the inflammatory response. The activated macrophages produced by LPS stimulation produce inflammatory mediators, such as nitric oxide (NO), TNF-α, eicosanoids, and cytokines and the inhibition of these are of prime importance for the prevention of inflammatory diseases (Ghayor, Gjoksi, Siegenthaler, & Weber, 2015). A recent investigation carried by Martinez-Villaluenga et al. (2012) documented that raw cabbage and sauerkraut have the ability to prevent production of nitric oxide by LPS-induced macrophages—both cabbage and sauerkraut produced by natural or induced fermentations suppressed NO production in a dose-dependent manner. However, sauerkraut was found to have higher ability to inhibit NO production as compared to raw cabbage.

9 Pickles

Pickling is one of the oldest gastronomic crafts of preserving foods using brine and/or vinegar. It has been an intrinsic process in all communities and cultures across the globe (Chakraborty & Roy, 2018). The production of fruit and vegetable, meat, and fish pickles has been an age-old tradition, but the ancient Mesopotamians also had to learn the art of preserving fruits and vegetables (Monika et al., 2017). Many ancient civilizations like ancient Egyptians, Indians, and Chinese used pickling as a method of preserving foods (Chakraborty & Roy, 2018).

Pickling is the preservation of foodstuffs by anaerobic fermentation under high acidic conditions, resulting in the formation of a product with a shelf life of over 2 years without refrigeration. Different types of pickles are prepared and have been divided on the basis of fermentation aspects into two categories (Behera, El Sheikha, Hammami, & Kumar, 2020):

1. Unfermented pickles: These are produced by two methods:
   a. Salt stock pickle: In this method, concentrated brine solution (up to 16% salt) is used and preservation is due to high salt concentration and not by fermentation.
   b. Vinegared: In this method, high acidic conditions are created by adding vinegar, and sometimes sugar is also added to help improve the flavor and texture.
2. Fermented pickles: These are of two types:
   a. Sour fermented: These pickles are prepared by submerging raw materials in a dilute brine of 2%–5% salt concentration. The growth of naturally occurring bacteria takes place over a period of 1–2 weeks to produce lactic acid that inhibits the growth of other undesirable microorganisms. The salt added controls the rate and type of fermentation also enhances the flavor.
   b. Sweet fermented: These pickles are preserved by a combination of lactic or acetic acid, sugar, and spices.

9.1 Production of pickles

Pickles are generally made from a mixture of different fruits and vegetables. They are served as a spicy condiment with the meal. The preservation of pickles occurs by a combination of high acidity, salt, reduced water activity, and added spices. Pickles are prepared by using one of two main methods: lactic acid fermentation of the food material with or without the addition of salt and the preservation of foodstuff in acetic acid (vinegar). The pickles made by these two methods differ significantly from each other in flavor and texture. Vegetables, including onion, cucumber, cabbage, and olives, are fermented by LAB that are able to grow in low salt concentration. These bacteria cause fermentation of sugars in the food to produce lactic acid, which results in the decrease in the pH of the food thereby preventing the growth of food poisoning and spoilage microorganisms (Noonari & Memon, 2015).

Raw materials such as fruits are first washed in clean running water and then peeled and the stones removed. The fruits used should be fresh and slightly underripe. Fruits that are damaged, bruised, or spoiled are removed. After this the fruits are sliced and/or diced into uniform-sized pieces using stainless steel knives. Accurate weighing devices are used so that the correct amount of ingredients is used. In the case of fermented pickles, the peeled fruits or vegetables are kept in brine (2%–3% salt solution) to prevent browning of the fruit or vegetable surfaces, while in the case of nonfermented pickles, vinegar, spices, and sugar are added to the mixture and then heated. Sometimes oil is added to the mixture. The vegetables are then place in a fermenter, to which salt or brine is added so that the vegetable pieces are submerged in it. The vegetables and salt are placed in alternate layers to fill three quarters of the container. Generally, 3 kg of salt is added to 100 kg vegetables. The salt added controls the type and rate of fermentation and also enhances the taste. Sometimes, a high level of salt (16%) is used to preserve the product by high salt concentration rather than by fermentation (Behera et al., 2020). The strength of the brine is measured using brine hydrometer or salometer.

Food materials like fruits and vegetables can be semiprocessed and preserved for several months by using high salt levels and can be then later processed into pickle. In order to increase the rate of fermentation, a starter culture from previous fermentation is added. The lid of the container is then closed to maintain an optimum temperature and to prevent the contamination of the product. The optimum temperature for fermentation is 21°C that should be carefully monitored to manufacture the product with desirable quality. Fermentation process starts as soon as the brine is formed, which becomes evident after the evolution of carbon dioxide gas bubbles produced by the bacteria. One to 4 weeks are usually required for fermentation, depending on the temperature, pH, and strength of the brine (Erten, 2016).

9.2 Health benefits of pickle

Fermented pickles have several important functions such as enhancement of flavors, alteration of textural characteristics, and improvement in the digestibility of foods. Many research investigations have documented that fermented pickles contain less amount of fat and cholesterol, which are rather health beneficial for humans. Moreover, the LABs (such as *L. lactis* and *S. thermophilus*) in pickles produce vitamins, such as riboflavin, cobalamin, folate, menaquinone, and thiamine during fermentation (LeBlanc et al., 2013). However, the pickle prepared from tropical roots and tubers is a fiber-rich product and has been found to improve the palatability and digestive quality of the foodstuffs. Furthermore, the fermented pickles have been found to have significant quantities of nutritious and bioactive compounds like proteins and amino acids and antioxidants, including phenols, flavonoids, and sterols (Frias et al., 2016; LeBlanc et al., 2013).

Pickling helps in the preservation and retention of natural bioactive compounds and antioxidants present in different food materials (Kübra, Alkan, & Kubra Sayin, 2015). Kübra et al. (2015) reported that high amount of proteins, vitamins, and dietary fibers was found in commercial pickled garlic when their physicochemical characterization was carried out. Pickled fruits and vegetables (colored) are rich source of anthocyanins, carotenoids, and flavonoids that scavenge free radicals produced due to different biological stresses and diseases in the body (Kübra et al., 2015). It has been reported by that Indian pickles contain significant high content of ascorbic acid that might inhibit benzene formation and associated cellular damage (Kharat, Adiani, Variyar, Sharma, & Singhal, 2016). Miller, Mack, and Knight (2009) documented that consumption of sufficient amount of pickle may help to relieve the muscle cramps of athletes by alteration electrolyte in plasma. Fermented pickles are a rich source of LABs that are "generally regarded as safe (GRAS)" and are the extensively studied microorganisms for probiotic traits because of their critical role in the regulation of the diverse gut microbiota and in the protection of human gastrointestinal tract (El Sheikha & Hu, 2020). In addition, fermented pickles with probiotic strains have been shown to play an important role in the prevention of colorectal cancer. It has been reported by Kumar et al. (2010) that the probiotic microbes may reduce the effects of proliferative and mutagenic activities and inhibit various enzymes involved in the generation of carcinogens, mutagens, and tumor-promoting agents.

The incidence of various lifestyle diseases like obesity and diabetes is reduced by consumption of fermented pickle as it has a high content dietary fiber. The diet containing probiotic strains (*L. casei* and *Lactobacillus acidophilus*) with low-fat pickle (2%–5%) is associated with the reduced risk of hyperinsulinemia, hyperglycemia, dyslipidemia, glucose intolerance, and oxidative stress, thereby lowering the risk of diabetes and its complications (Fujii, Watanabe, Horikoshi, Takahashi, & Kimura, 2011; Yadav, Jain, & Sinha, 2007).

10 Idli

Idli is very popular fermented snack or breakfast food consumed mainly in southern India and Sri Lanka. It is prepared by fermentation of rice (*Oryza sativa* L.), especially parboiled and decorticated black gram (*Phaseolus mungo* L.) dal (Rani, Amane, & Ananthanarayan, 2019). It is highly nutritious as it is prepared from a blend of carbohydrate-rich cereal and protein-rich legume. Legumes and cereals when consumed together act as a source of functional proteins with a balanced essential amino acid profile (Amane & Ananthanarayan, 2018).

10.1 Production of idli

Idli, made from black gram dehulled dal and rice (3:1), is a time-consuming process. Initially, rice and black gram dal are soaked in water for 4 hours and then ground separately. Rice and black gram dal are then mixed to form a fine batter, followed by addition of salt and then allowed to ferment at 30°C ± 2°C for about 12 hours. The fermented batter is dispensed in special idli pans or molds and steamed for about 5–8 minutes to form small idlis (Kumari, Bhinder, Singh, Kaur, & Singh, 2020; Mulmule et al., 2017). During fermentation the naturally occurring microorganisms, including *L. mesenteroides* and *Streptococcus thermophiles*, grow rapidly in idli batter as compared to the initial contaminants and thus dominating the fermentation. These microorganisms are responsible for the production of lactic acid (≥1%) and carbon dioxide, which make the idli batter anaerobic and leaven the product (Ghosh & Chattopadhyay, 2011).

10.2 Nutritional profile and health benefits

Nutritional composition of idli varies significantly depending on the type of idlis (prepared by varying the proportions of black gram and rice). The protein, carbohydrate, fat, and crude fiber contents of the idli have been found to vary in between 7.0 and 7.2 g/100 g, 52.10 and 75.6 g/100 g, 0.54 and 0.84 g/100 g, and 0.62 and 1.08 g/100 g, respectively, with an energy value of about 241.94–328.19 kcal. It is also a good source of calcium and iron (Krishnamoorthy, Kunjithapatham, & Manickam, 2013; Nazni & Shalini, 2020). Most of the cereals are deficient in lysine and rich in sulfur-containing amino acids (cysteine and methionine). However, legumes are rich in lysine but are poor in cysteine and methionine. Idli, therefore, boosts a relatively good nutritional profile owing to the type of ingredients present in it (Kumari et al., 2020). Considering the energy requirements of children and women who lack a proper diet, idli can play a crucial role as supplementary food.

Fermentation of idli batter improves its vitamin B, vitamin C, iron and zinc content (Sahoo & Author, 2017). It has been found that fermented idli helps in reducing the weight and thus decreases risk of obesity. In addition, consumption of idli is associated with the reduced risk of CVDs, stroke, and high blood pressure. The children suffering from protein kwashiorkor can be given idli as a dietary supplement to prevent protein and calorie malnutrition. Idli also acts as source of micronutrients, including calcium, folate, zinc, and iron, that provide nourishment to the muscle and bone, help prevent

anemia, and facilitate the oxygenation of blood. Idli has improved digestibility and high dietary fiber content that gives bulk stool feelings and reduces constipation (Mulmule et al., 2017).

11 Miso

Miso is a Japanese traditional fermented product used as a condiment for soups and vegetable dishes. It is consumed as a breakfast by most of the Japanese families from over 1000 years. Many varieties of miso have been prepared in Japan based on the local traditions and the types of ingredients available or the desirability of the product (Machida, Yamada, & Research, 2008). Generally, miso is prepared by using soybeans, salt, and different types of koji (steamed grains inoculated with *Aspergillus oryzae*). Miso can be classified into three types on the basis of koji used: (1) rice miso, prepared by adding rice koji to soybeans, has a prominent aroma of rice; (2) barley miso, prepared by adding barley koji to soybeans, rich in flavor with barley aroma; (3) soy miso, made from soybeans only, has moderate sweetness and gives prominent fragrance even during cooking (Kusumoto & Rai, 2017; Wang et al., 2019). Rice miso is the most common type of miso and accounts for 80% of the total miso production (Kitamura et al., 2016). It is further subdivided into six types depending on the taste and color, namely, red salty, yellowish salty, red sweet, white sweet, brown, and white. Of these, red salty miso is the most popular in northern parts of Japan (Wang et al., 2019).

11.1 Production of miso

Miso can be prepared either by adding rice or barley. The rice or barley is cooked for about 40 minutes and the soybeans are soaked and then cooked for 15 minutes at 115°C. The rice or barley is inoculated with koji mold, *A. oryzae*, and incubated at 35°C–38°C for 40–48 hours to promote amylase production instead of protease. After this, salt is added to prevent further mold growth and then the salted barley or rice koji is mixed with cooked soybeans. Sometimes, LAB and yeast may also be added. The mixture is then transferred to a tank and the lid is closed and incubated at 30°C for two more months (Uraz, 2014). During aging, it is transferred from one tank to another to properly mix the contents and to incorporate some oxygen required for mold growth. After aging is complete the miso is blended, mashed, and pasteurized, followed by addition of 2% alcohol to inhibit yeast (Mani & Ming, 2017; Wang et al., 2019).

11.2 Nutritional profile and health benefits

Miso is a protein-rich paste and is highly popular as it provides instant flavor foundation. It is consumed as condiment and adds fifth taste known as umami to all types of soups and vegetable dishes. Miso is a rich source of minerals, including copper manganese and zinc, and B-complex vitamins, vitamin E, K, and folic acid. Since miso is a fermented product, it increases the number and growth of beneficial bacteria in the gut, thereby improving human health. These bacteria, known as probiotics help to improve digestion, absorption, and assimilation of nutrients in humans with wide range of health issues. Miso is a soybean-based food that is a good source of isoflavones that confer significant health benefits. The presence of isoflavones in soybean-based foods and their role against carcinogenesis has attracted worldwide attention. Bolla (2015) documented that isoflavones can delay the onset of certain types of cancer. The major isoflavones present in soybeans are genistein and daidzin constituting about 99% of the total concentration (Fukutake et al., 1996). Genistein has been found to be more effective in preventing the cell growth of human prostate cancer cells in comparison to daidzin (Onozawa et al., 1998). It has been reported that soybean-based fermented foods contain high concentration of genistein than unfermented foods (Cheng, Daidzin, & After, 2001).

Miso and its constituents have been found to be associated with reduced blood pressure, inhibition of osteoporosis, prevention of heart diseases and cancer, and decreasing the risk of degenerative diseases. Okouchi, Sakanoi, and Tsuduki (2019) reported that accumulation of lipids in the adipose tissues is suppressed by miso consumption in combination with exercise. Miso, due to its high antioxidant activities, can play a critical role in preventing aging caused by oxidative stress. These health benefits make miso a popular functional food in different parts of the world, especially in Japan (Tatsuya et al., 2013).

12 Yoghurt

Yoghurt is a fermented milk product obtained by the lactic acid fermentation of milk or the milk products by the action of two species of LAB, that is, *Streptococcus salivarius* subsp. *thermophilus*, and *L. delbrueckii* subsp. *bulgaricus*. The fermentation of milk results in the production of sufficient quantity of lactic acid leading to milk coagulation and this product is known as yoghurt.

The term "yoghurt" has been from Turkish word "jugurt," used to describe any fermented food with an acidic taste (Younus, Masud, & Aziz, 2002). Yoghurt appeared the years 9000 or 8000 BCE in Mesopotamia and Egypt and gradually spread to northeast of Africa, Middle East, and Central Asia and then later to Balkan countries. Yoghurt originally resulted from accidental spontaneous lactic acid fermentation of milk, which led to its acidification and coagulation, thereby preserving the raw material effectively which otherwise could spoil rapidly. It was first produced commercially in Barcelona in 1919 by Isaac Carasso (Corrieu & Béal, 2017).

12.1 Production of yoghurt

Yoghurt manufacture at industrial level consists of three main steps:

1. Preparation of the mix and its standardization, pasteurization, cooling, and deaeration
2. Fermentation after addition of starter culture
3. Yoghurt harvesting, posttreatments, and packaging

Four types of yoghurts can be prepared by this method. Each step is very important in the yoghurt manufacture and affects the quality of the final product. The product flavoring and cup filling are done after fermentation for each product, except for set-type yoghurts (Cn, Vu, & Cp, 2016).

1. **Preparation of the mix**
   a. **Standardization of milk**
      In order to obtain a milk of uniform quality, standardization process of the mix is performed. The standardization mainly involves protein and fat contents and sometimes addition of sweeteners and stabilizers. Fat is standardized by centrifuging it at about 55°C, followed by cream addition to attain the desired fat content. Generally, the fat content ranges from nonfat (0.01%), to low- or light-fat (1%–2%), to whole-fat yogurts (>3.2%). The standardization of protein is carried to increase its content in the mix from 3% to 5%–15% in order to improve the texture of yoghurt and decrease its syneresis. It is usually done by adding dry milk powder to the mix. Milk proteins or milk replacers as caseinates or whey powders are also commonly used. It is recommended that dry ingredients are mixed completely without air incorporation. Concentration by ultrafiltration and reverse osmosis is another method to increase the amount of protein in the mix. In some cases, sugar or other sweeting agent is added to the mix. The use of thickeners and stabilizers, including gelatin, pectin, xanthan gum, carrageenan, and starch, at concentrations varying from 5% to 10% is permitted in some countries by FAO/WHO for improving the yoghurt texture.
   b. **Physical treatments of the mix (pasteurization, cooling, and deaeration)**
      Heating is an important step in the preparation of mix. It results in the destruction of spoilage microorganisms, inactivation of enzymes, and production of stimulatory compounds. At industrial scale, yoghurts are generally heated at 90°C or 95°C for 3–7 minutes and then cooled down to fermentation temperature (37°C–43°C) (Tamime & Robinson, 1994).
      The other two treatments given to the mix are deaeration and homogenization that are closed associated with the heat treatment. Homogenization is done to obtain a product with uniform quality and also to increase the gel texture and decrease syneresis. It results in the reduction of size of the fat globules and improves the association of between fat and hydrophilic proteins. Air removal or deaeration of the mix is done in order to remove the oxygen content and thus reduce the fermentation time, improve the texture of the yoghurt, and remove off-flavors. Deaeration is normally done at 70°C, before homogenization.
2. **Fermentation process**
   a. **Addition of inoculum to the mix**
      Commercially, yoghurts are prepared by inoculating the mix with starter cultures of two bacteria (*S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*). These starter cultures consist of specific blends of selected and well-defined strains, at a concentration more than 10^{10} CFU/g. This inoculated mix generally contains 10^6–10^7 CFU/mL of bacteria. It is then transferred to fermentation tanks after mixing it properly (for stirred-type yoghurt) or directly to the packaging machine for fermentation in cups for set-type yoghurt (Banon, Vetier, & Hardy, 2009).
   b. **Fermentation step**
      Lactic acid fermentation of milk involves several parameters that vary as a function of time. Initially, the growth of *S. thermophiles* occurs, followed by *L. bulgaricus*, reaching final concentrations to nearly 10^9 CFU. These bacteria consume lactose and nitrogenous compounds during growth and lead to the accumulation of many relevant metabolites. The most important ones produced which contribute to the flavor and texture of yoghurt include lactic acid, galactose, acetaldehyde, and exopolysaccharides. Acetaldehyde is responsible for the characteristic aroma of yogurt

and exopolysaccharides enhance its texture. The acidification process of the yoghurt is controlled by the final pH of the yogurt and the acidification rate, which are critical for the quality of the product. The fermentation process is then stopped rapidly by cooling the product when the final pH has reached which varies from 4.5 to 4.8. The rate of acidification process is influenced by numerous factors such as inoculum composition and activity, composition of mix, physical treatments, and fermentation temperature. However, to maintain the accurate temperature is not possible during yoghurt fermentation as the coagulation phenomenon occurs at pH 5.5. Consequently, the fermentation time may vary. It has been found that the fermentation time for probiotic yoghurt can reach 6–8 hours, while for stirred yoghurt is required (Corrieu & Béal, 2017).

3. **Harvesting of yoghurt and packaging**
   a. **Cooling and harvesting of yogurt**
      The first step of yoghurt harvesting is the rapid cooling of the product to stop its further acidification. It is done after the desired pH of the yoghurt has reached (Tamime & Deeth, 1980). Set-type yoghurts are cooled to 4°C–5°C within 1 or 2 hours by using cold air–ventilated cabinets, cooling rooms, or tunnels. Stirred-type yoghurts are cooled to a temperature between 18°C and 25°C in less than 1 hour by using an external heat exchanger.
   b. **Packaging of yogurt**
      The yoghurt is finally packed to ensure its hygiene and protection during distribution. Generally, plastic and glass cups are used for the packaging of set-type yoghurts. However, large-up-date packaging units use form-fill-seal technology. After packaging the product is stored under refrigeration (4°C–5°C), which reduces its postacidification and ensures quality and safety of the product (Macbean, 2010).

12.2 Nutritional profile and health benefits

Milk contains macronutrients that are well balanced between protein, fat, and carbohydrates. It is also a good source of a wide range of micronutrients including calcium, phosphorous, magnesium, and zinc (Banon, Vetier, & Hardy, 2009). The composition of yoghurt depends on the tastes of the market and the specific segment in the market. The fat content of the milk may vary from zero (yoghurts made from skim milk) to higher than 4% (indulgence products) and sometimes up to 8% or 9% (Greek-style products). The protein content may remain same as those of the milk from which the yoghurt is prepared or significantly higher to enhance the yoghurt firmness. Yogurt contains the nutrients of the milk, that is, fat, protein, lactose, and minerals and vitamins, as well as other nutrients, and is thus widely recognized as a nutritious food product. The average values for total solids (TS), solid non fat, fat, protein, and ash contents of the yoghurt have been found in between 10.57% and 12.22%, 12% and 15%, 3.04% and 3.53%, 2.93% and 4.42%, and 0.66% and 0.89%, respectively (El-zubeir, 2014; Oliveira, Telles, Fernandes, & Balian, 2002).

Yoghurt has been found to have numerous health benefits. It has been documented to reduce medical conditions by restoring the beneficial microorganisms in the colon, reducing constipation and diarrhea. The yoghurt prepared from cow milk has low fat and thus boosts immunity and provides protection against cold, cough and improves defense mechanism of the body. Further, it helps to lower blood pressure, bad cholesterol, and risk of heart attack. Yoghurt is rich in calcium and thus protects the bones from osteoporosis and arthritis. By regular consumption of yoghurt, disease-causing bacteria are flushed out from the colon that prevents the occurrence of colon cancer (Banon, Vetier, & Hardy, 2009; Corrieu & Béal, 2017; McKinley, 2005).

13 Dosa

In Indian subcontinent, fermented foods like pickles, dairy products, for example, dahi, yoghurt, cereal-based foods form human body, and to achieve good nutrition, various sources of energy are harvested. Cereals in general and millets in particular offer a cheap source of nutrients and energy source as compared to the staple sources of energy like rice and wheat. Whole millets and millets products are widely consumed across the globe and in the Indian subcontinent the products derived from millets are especially consumed as traditional breakfast cereals in South India. However, due to various antinutrient factors like phytates, phenols, tannins trypsin inhibitors, etc. that are present in millets, the maximum harvesting of the nutrient potential becomes limited to a certain extent. Among different traditional heritage fermented foods of India that are mainly based on cereals mainly on rice and millets are idli and dosa. Dosa originated as a traditional breakfast cereal from South India and nature of the food is mainly cereal and legume based and shares its importance as a traditional breakfast cereal at par with idli. Dosa is usually consumed in the form of a crispy pancake made on hot tawa. Vegetable mixtures are wrapped inside the pancake as a filling and then rolled like a chicken roll. Different recipes have been developed with time in accordance with taste and regional profile. Traditionally, across the Indian subcontinent, the recipe

consists of rice and lentils. The usual method of preparation follows as three to four parts of rice is mixed with black gram that may be either raw or parboiled and salt is added according to taste. The usual procedure involves the following steps: first, the raw ingredients are soaked in plain water and as the rice and dhal are slightly softened, they are finely ground and added with a certain quantity of water until smooth consistency is obtained. The smooth batter is collected in a vessel and incubated at room temperature for a period of almost 24 hours. After the fermentation process is complete, the consistent batter is spread in the form of a thin layer on a hot flat tawa that is initially smeared with ghee or edible oil. The pancake is stuffed with vegetables and consumes as a snack. Dosa fermentation involves a number of microbial colonies associated with the fermentation of rice starch and black gram. The majority of the fermenting microbial communities comprise LAB such as *L. mesenteroides*, *S. faecalis*, *Lactobacillus fermentum*, *Bacillus amyloliquefaciens*, *L. lactis*, *L. delbrueckii*, and *L. plantarum*. Other minor microbes that are predominantly yeasts are also involved in dosa fermentation like *S. cerevisiae*, *D. hansenii*, *Trichosporon beigelii*, *Torulopsis* spp., and *Trichosporon* spp (Blandino et al., 2003; Kumar et al., 2013). During the fermentation process the nutrient profile of dosa is considerably increased. The biochemical profile of dosa reveals the presence of different compounds of considerable importance such as organic acids, TS, nitrogenous compounds, enzymes, vitamins, and various pigments. A good quantity of antinutritional compounds is reduced during the process of fermentation that in turn results in bioaccessibility of bound nutrients and minerals especially zinc and iron (Gupta & Tiwari, 2014). For individuals suffering from allergic syndromes like gluten intolerance or wheat intolerance, dosa offers to be an appropriate alternative to chapatis. The added nutrient profile of black gram dhal supports the low glycemic index of dosa and helps to control glucose metabolism in the case of diabetic conditions (Gupta & Tiwari, 2014). The cereal-based products, that is, idli and dosa contain substrates for the growth of different microbial colonies especially *Lactobacilli* and *Bifidobacteria* that are mainly involved in the fermentation process.

13.1 Health benefits of dosa

Dosa is said to be a complex medium of various microbial communities, out of which the predominant yeast species are *Saccharomyces*, *Debaryomyces*, *Trichosporon*, and *Hansenula*, while as *Leuconostoc* and *Lactobacillus* are the main bacterial species involved in the fermentation process of the final dosa batter. The yeast species are involved in the production of majority of the amylolytic enzymes that are in turn involved in the breakdown of complex carbohydrate matrix, proteins, etc. to yield simple sugars, bioactive peptides, and vitamins involved in various health-promoting processes.

14 Conclusion

Nowadays, the production and consumption of traditional fermented foods have gained momentum across the globe. Traditional foods play a very important role in maintain harmony across the social sectors and hold economies of various regions intact and as such are recognized as an important potential in combating malnutrition because of their huge nutritional potential. The majority of the probiotic fermented foods represent every community across the world with its unique process of production and its nutritional value. Different fermented foods mentioned previously contain a vast majority of microbial communities that ultimately contribute to the overall probiotic potential of the foods. The microbial communities of all the food systems mentioned previously have been well defined and characterized. The biochemical profile of all the probiotic foods shows a myriad quantity of both biological and nutraceutical potential. The nutritional aspects cover a vast range of health benefits like deciphering anticancer properties, antioxidant properties, hypolipidemic activities, etc. The probiotics possess functional attributes and fulfill the nutritional and supplementary requirements of the whole population concerns. The focus of the chapter was to elaborate whole of the manufacturing process with main focus on nutritional profile and nutraceutical properties in order to emphasize their consumption for benefaction to human health.

References

Adiloğlu, A. K., Gönülateş, N., & İşler, M. (2013). The effect of kefir consumption on human ımmune system: A cytokine study. *Mikrobiyoloji Bülteni*, *47*(2), 273–281.

Aggarwal, B. B., & Shishodia, S. (2006). Molecular targets of dietary agents for prevention and therapy of cancer. *Biochemical Pharmacology*, *71*, 1397–1421.

Ahmad, A., Ramasamy, K., Jaafar, S. M., Majeed, A. B., & Mani, V. (2014). Total isoflavones from soy-bean and tempeh reversed scopolamine-induced amnesia, improved cholinergic activities and reduced neuroinflammation in brain. *Food and Chemical Toxicology*, *65*, 120–128.

Ahmad, A., Ramasamy, K., Majeed, A. B., & Mani, V. (2015). Enhancement of beta-secretase inhibition and antioxidant activities of tempeh, a fermented soybean cake through enrichment of bioactive aglycones. *Pharmaceutical Biology*, *53*, 758–766.

Ahmed, Z., Wang, Y., & Ahmad, A. (2013). Health: A contemporary perspective. *Critical Reviews in Food Science and Nutrition*, *53*(5), 422–434.

Alp, D., & Ertürkmen, P. (2017). Used as a probiotic *Lactobacillus* spp. strains of cholesterol lowering effects and possible mechanisms. *The Journal of Graduate School of Natural and Applied Sciences of Mehmet Akif Ersoy University*, *8*(1), 108–113.

Amane, D., & Ananthanarayan, L. (2018). Quantification of rice and black gram dal proportions in idli batters by estimation of starch, daidzein and trypsin inhibitor activity. *LWT—Food Science and Technology*, *98*(June), 622–628. https://doi.org/10.1016/j.lwt.2018.09.038.

Anderson, J., & Gilliland, S. E. (1999). Effect of fermented milk (yogurt) containing *Lactobacillus acidophilus* L1 on serum cholesterol in hypercholesterolemic humans. *Journal of American Collection of Nutrition*, *18*, 43–50.

Angulo, L., Lopez, E., & Lema, C. (1993). Microflora present in Kefir grains of Galician region (North-West of Spain). *Journal of Dairy Research*, *60*, 263–267.

Arroyo-Lopez, F. N., Perez-Traves, L., Querol, A., & Barrio, E. (2011). Exclusion of *Saccharomyces kudriavzevii* from a wine model system mediated by *Saccharomyces cerevisiae*. *Yeast*, *28*, 423–435.

Arslan, S. (2015). A review: Chemical, microbiological and nutritional characteristics of kefir. *CYTA—Journal of Food*, *13*, 340–345.

Assadi, M. M., Pourahmad, R., & Moazami, N. (2000). Use of isolated kefir starter cultures in kefir production. *World Journal of Microbiology and Biotechnology*, *16*(6), 541–543.

Ayed, L., & Hamdi, M. (2015). Manufacture of a beverage from cactus pear juice using "tea fungus" fermentation. *Annals of Microbiology*, *65*(4), 2293–2299.

Balentine, D. A., Wiseman, S. A., & Bouwens, L. C. (1997). The chemistry of tea flavonoids. *Critical Reviews in Food Science and Nutrition*, *37*, 693–704.

Banon, S. D., Vetier, N., & Hardy, J. (2009). Health benefits of yogurt consumption. A review. *International Journal of Food Properties*, *2912*(1999), 1–12. https://doi.org/10.1080/10942919909524585.

Battikh, H., Bakhrouf, A., & Ammarb, E. (2012a). Antimicrobial effect of *Kombucha analogues*. *Journal of Food Science and Technology*, *47*, 71–77.

Battikh, H., Bakhrouf, A., & Ammar, E. (2012b). Antimicrobial effect of kombucha analogues. *LWT—Food Science and Technology*, *47*(1), 71–77.

Beganović, J., Kos, B., Leboš Pavunc, A., Uroić, K., Jokić, M., & Šušković, J. (2014). Traditionally produced sauerkraut as source of autochthonous functional starter cultures. *Microbiological Research*, *169*(7–8), 623–632. https://doi.org/10.1016/j.micres.2013.09.015.

Behera, S. S., El Sheikha, A. F., Hammami, R., & Kumar, A. (2020). Traditionally fermented pickles: How the microbial diversity associated with their nutritional and health benefits? *Journal of Functional Foods*, *70*(December 2019), 103971. https://doi.org/10.1016/j.jff.2020.103971.

Bhattacharya, S., Manna, P., Gachhui, R., & Sil, P. C. (2011). Protective effect of kombucha tea against tertiary butyl hydroperoxide induced cytotoxicity and cell death in murine hepatocytes. *Indian Journal of Experimental Biology*, *49*, 511–524.

Bhattacharya, S., Gachhui, R., & Sil, P. C. (2013). Effect of kombucha, a fermented black tea in attenuating oxidative stress mediated tissue damage in alloxan induced diabetic rats. *Food and Chemical Toxicology*, *60*, 328–340.

Blanc, P. J. (1996a). Characterization of the tea fungus metabolites. *Biotechnology Letters*, *18*, 139–142.

Bolla, K. N. (2015). Soybean consumption and health benefits. *International Journal of Scientific & Technology Research*, *4*(7), 50–53.

Bong, Y. J., Jeong, J. K., & Park, K. Y. (2013). Fermentation properties and health functionality improvement of kimchi by kimchi lactic acid bacteria starters. *Journal of the Korean Society of Food Science and Nutrition*, *42*, 1717–1726.

Borresen, E. C., Henderson, A. J., Kumar, A., Weir, T. L., & Ryan, E. P. (2012). Fermented foods: Patented approaches and formulations for nutritional supplementation and health promotion. *Recent Patents on Food, Nutrition & Agriculture*, *4*, 134e40.

Bourrie, B. C. T., Willing, B. P., & Cotter, P. D. (2016). The microbiota and health promoting characteristics of the fermented beverage kefir. *Frontiers in Microbiology*, *7*, 1–17.

Breidt, F., Crowley, K. A., & Fleming, H. P. (1995). Controlling cabbage fermentations with nisin and nisin-resistant *Leuconostoc mesenteroides*. *Food Microbiology*, *12*(C), 109–116. https://doi.org/10.1016/S0740-0020(95)80086-7.

Can, G., Topuz, E., & Derin, D. (2009). Effect of kefir on the quality of life of patients being treated for colorectal cancer. *Oncology Nursing Forum*, *36*(6), 335–342.

Carroll, K. K. (1991). Review of clinical studies on cholesterol-lowering response to soy protein. *Journal of the American Dietetic Association*, *91*, 820–827.

Cetojevic-Simin, D. D., Bogdanovic, G. M., Cvetkovic, D. D., & Velicanski, A. S. (2008). Antiproliferative and antimicrobial activity of traditional kombucha and *Satureja montana* L. kombucha. *Journal of Balkan Union of Oncology*, *13*, 395–401.

Chakraborty, R., & Roy, S. (2018). Exploration of the diversity and associated health benefits of traditional pickles from the Himalayan and adjacent hilly regions of Indian subcontinent. *Journal of Food Science and Technology*, *55*(5), 1599–1613. https://doi.org/10.1007/s13197-018-3080-7.

Chakravorty, S., Bhattacharya, S., Chatzinotas, A., Chakraborty, W., Bhattacharya, D., & Gachhui, R. (2016). Kombucha tea fermentation: Microbial and biochemical dynamics. *International Journal of Food Microbiology*, *220*, 63–72.

Chang, J. H., Shim, Y. Y., Cha, S. K., & Chee, K. M. (2010). Probiotic characteristics of lactic acid bacteria isolated from kimchi. *Journal of Applied Microbiology*, *109*, 220–230.

Cheigh, H. S., & Park, K. Y. (1994). Biochemical, microbiological and nutritional aspect of kimchi. *Critical Reviews in Food Science and Nutrition*, *43*, 175–203.

Chen, Y. -S., Yanagida, F., & Hsu, J. -S. (2006). Isolation and characterization of lactic acid bacteria from suan-tsai (fermented mustard), a traditional fermented food in Taiwan. *Journal of Applied Microbiology*, *101*(1), 125–130.

Cheng, L., Daidzin, C., & After, C. (2001). Isoflavone transformation during soybean koji preparation and subsequent miso fermentation supplemented with ethanol and NaCl. *Journal of Agricultural and Food Chemistry*, *49*, 3656–3660. https://doi.org/10.1021/jf0015241.

Cho, E. J. (1999). *Standardization and cancer chemopreventive activities of Chinese cabbage kimchi*. (pp. 625–632) Busan, Korea: Pusan National University. Ph.D. Thesis.

Choi, J. W., Kang, H. W., & Lim, W. C. (2017). Kefir prevented excess fat accumulation in diet induced obese mice. *Bioscience, Biotechnology, and Biochemistry*, *81*(5), 958–965.

Cn, O., Vu, O., & Cp, M. (2016). Yoghurt production from powdered milk using mixed lactic acid bacteria starter cultures. *Original Research Article Saudi Journal of Pathology and Microbiology*, 1242–1249. https://doi.org/10.21276/sjpm.2016.1.2.3.

Corrieu, G., & Béal, C. (2017). Yogurt: The product and its manufacture. In *Encyclopedia of food and health*In (5). (1st ed., pp. 617–624). Elsevier Ltd.. Issue July 2016, https://doi.org/10.1016/B978-0-12-384947-2.00766-2.

Coton, M., Pawtowski, A., Taminiau, B., Burgaud, G., Deniel, F., Coulloumme-Labarthe, L., et al. (2017). Unraveling microbial ecology of industrial-scale Kombucha fermentations by metabarcoding and culture-based methods. *FEMS Microbiology Ecology*, *93*. https://doi.org/10.1093/femsec/fix048.

Da Cruz Pedrozo Miguel, M. G., Cardoso, P. G., De Assis Lago, L., & Schwan, R. F. (2010). Diversity of bacteria present in milk kefir grains using culture-dependent and culture-independent methods. *Food Research International*, *43*, 1523–1528.

Dani, A. H., & Masson, V. M. (1996). *History of civilizations of Central Asia: The dawn of civilization: Earliest times to 700 BC* (38, pp. 283–288). UNESCO.

Danielian, L. T. (2005). *Kombucha and its biological features*. Moscow: Meditsina.

Di Cagno, R., Coda, R., De Angelis, M., & Gobbetti, M. (2013). Exploitation of vegetables and fruits through lactic acid fermentation. *Food Microbiology*, *33*(1), 1–10. https://doi.org/10.1016/j.fm.2012.09.003.

Donath, M. Y. (2014). Targeting inflammation in the treatment of type 2 diabetes: Time to start. *Nature Reviews Drug Discovery*, *13*(6), 465–476. https://doi.org/10.1038/nrd4275.

Dong, X., Xu, W., Sikes, R. A., & Wu, C. (2013). Combination of low dose of genistein and daidzein has synergistic preventive effects on isogenic human prostate cancer cells when compared with individual soy isoflavone. *Food Chemistry*, *141*, 1923–1933.

Dong-Hyeon, K., Hyunsook, K., & Dana, J. (2017). Kefir alleviates obesity and hepatic steatosis in high-fat diet-fed mice by modulation of gut microbiota and mycobiota: Targeted and untargeted community analysis with correlation of biomarkers. *Journal of Nutritional Biochemistry*, *44*, 35–43.

Droge, W. (2002). Free radicals in the physiological control of cell function. *Physiological Reviews*, *82*, 47–95.

Duffy, R., Wiseman, H., & File, S. E. (2003). Improved cognitive function in postmenopausal women after 12 weeks of consumption of a soya extract containing isoflavones. *Pharmacology Biochemistry and Behavior*, *75*, 721–729.

Dufresne, C., & Farnworth, E. (2000). Tea, kombucha, and health: A review. *Food Research International*, *33*(6), 409–421.

Eales, J., Gibson, P., Whor well, P., Kellow, J., Yellowlees, A., Perry, R. H., et al. (2017). Systematic review and meta-analysis: The effects of fermented milk with *Bifidobacterium lactis* CNCM I-2494 and lactic acid bacteria on gastrointestinal discomfort in the general adult population. *Therapeutic Advances in Gastroenterology*, *10*, 74–88.

El Sheikha, A. F., & Hu, D. M. (2020). Molecular techniques reveal more secrets of fermented foods. *Critical Reviews in Food Science and Nutrition*, *60*(1), 11–32. https://doi.org/10.1080/10408398.2018.1506906.

Ellulu, M. S., Rahmat, A., Patimah, I., Khaza'Ai, H., & Abed, Y. (2015). Effect of vitamin C on inflammation and metabolic markers in hypertensive and/or diabetic obese adults: A randomized controlled trial. *Drug Design, Development and Therapy*, *9*, 3405–3412. https://doi.org/10.2147/DDDT.S83144.

El-zubeir, I.E.M. (2014). Chemical composition and microbial load of set yoghurt from fresh and recombined milk powder in Khartoum State. August. https://doi.org/10.3923/ijds.2011.172.180.

Farnworth, E. R. (2005). Kefir—A complex probiotic. *Food Science & Technology Bulletin: Functional Foods*, *2*(1), 1–17.

Fathi, Y., Faghih, S., & Zibaeenezhad, M. J. (2016). Kefir drink leads to a similar weight loss, compared with milk, in a dairy-rich non-energy-restricted diet in overweight or obese premenopausal women: A randomized controlled trial. *European Journal of Nutrition*, *55*, 295–304.

Fernandes, J. V., Cobucci, R. N. O., Jatobá, C. A. N., de Medeiros Fernandes, T. A. A., de Azevedo, J. W. V., & de Araújo, J. M. G. (2015). The role of the mediators of inflammation in cancer development. *Pathology and Oncology Research*, *21*(3), 527–534. https://doi.org/10.1007/s12253-015-9913-z.

Franco, M. C., Golowczyc, M. A., & De Antoni, G. L. (2013). Administration of kefir-fermented milk protects mice against Giardia intestinalis infection. *Journal of Medical Microbiology*, *62*, 1815–1822. https://doi.org/10.1099/jmm.0.068064-0.

Frengova, G. I., Simova, E. D., Beshkova, D. M., & Simov, Z. I. (2002). Exopolysaccharides produced by lactic acid bacteria in kefir grains. *Zeitschrift für Naturforschung*, *57*, 805–810.

Frias, J., Martinez-Villaluenga, C., & Peñas, E. (2016). *Fermented foods in health and disease prevention*. Elsevier Inc.. https://doi.org/10.1016/c2014-0-01734-0.

Fujii, T., Watanabe, S., Horikoshi, M., Takahashi, H., & Kimura, B. (2011). PCR-DGGE analysis of bacterial communities in funazushi, fermented crucian carp with rice, during fermentation. *Fisheries Science*, *77*(1), 151–157. https://doi.org/10.1007/s12562-010-0306-7.

Fukutake, M., Takahashi, M., Ishida, K., Kawamura, H., Sugimura, T., & Wakabayashi, K. (1996). Quantification of genistein and genistin in soybeans and soybean products. *Food and Chemical Toxicology*, *34*(5), 457–461. https://doi.org/10.1016/0278-6915(96)87355-8.

Furukawa, S., Watanabe, T., Toyama, H., & Morinaga, Y. (2013). Significance of microbial symbiotic coexistence in traditional fermentation. *Journal of Bioscience and Bioengineering*, *116*, 533–539.

Gharib, O. A. (2014). Effect of kombucha on some trace element levels in different organs of electromagnetic field exposed rats. *Journal of Radiation Research and Applied Sciences*, *7*(1), 18–22.

Ghayor, C., Gjoksi, B., Siegenthaler, B., & Weber, F. E. (2015). *N*-Methyl pyrrolidone (NMP) inhibits lipopolysaccharide-induced inflammation by suppressing NF-κB signaling. *Inflammation Research*, *64*(7), 527–536. https://doi.org/10.1007/s00011-015-0833-x.

Ghosh, D., & Chattopadhyay, P. (2011). Preparation of idli batter, its properties and nutritional improvement during fermentation. *Journal of Food Science and Technology*, *48*(5), 610–615. https://doi.org/10.1007/s13197-010-0148-4.

Goh, W. N., Rosma, A., Kaur, B., Fazilah, A., Karim, A. A., & Bhat, R. (2012). Fermentation of black tea broth (kombucha): I. Effects of sucrose concentration and fermentation time on the yield of microbial cellulose. *International Food Research Journal*, *19*(1), 109–117.

Gorcia Hermosilla, J. A., Jha, H. M., Egge, H., Mahmud, M., Hermana, S., & Rao, G. S. (2014). Isolation and characterization of hydroxymethylglutaryl Coenzyme A reductase inhibitors from fermented soybean extracts. *J Clin BiochemNutr*, *122*, 163–174.

Grishina, A., Kulikova, I., & Alieva, L. (2011). Antigenotoxic effect of kefir and ayran supernatants on fecal water-induced DNA damage in human colon cells. *Nutrition and Cancer*, *63*(1), 73–79.

Gupta, A., & Tiwari, S. K. (2014). Probiotic potential of *Lactobacillus plantarum* LD1 isolated from batter of dosa, a South Indian fermented food. *Probiotics Anti-microb Proteins*, *6*, 73–81.

Han, G. J., Son, A. R., Lee, S. M., Jung, J. K., Kim, S. H., & Park, K. Y. (2009). Improved quality and increased in vitro anticancer effects of kimchi by using natural sea salt without bittern and baked (Guwun) salt. *Journal of the Korean Society of Food Science and Nutrition*, *38*, 996–1002.

Hara, T., Hiroyuki, S., Nobuhide, I., & Shinji, K. (1995). Plasmid analysis in polyglutamate-producing *Bacillus* strain isolated from non-salty fermented soybean food, "kinema", in Nepal. *Journal of General and Applied Microbiology*, *41*, 3–9.

Hayes, J. D., Kelleher, M. O., & Eggleston, I. M. (2008). The cancer chemopreventive actions of phytochemicals derived from glucosinolates. *European Journal of Nutrition*, *47*(Suppl. 2), 73–88. https://doi.org/10.1007/s00394-008-2009-8.

Hogervorst, E., Mursjid, F., Priandini, D., Setyawan, H., Ismael, R. I., Bandelow, S., et al. (2011). Borobudur revisited: Soy consumption may be associated with better recall in younger, but not in older, rural Indonesian elderly. *Brain Research*, *1379*, 206–212.

Hur, H. J., Lee, K. W., & Lee, H. J. (2004). Production of nitric oxide, tumor necrosis factor-α and interleukin-6 by RAW 264.7 macrophage cells treated with lactic acid bacteria isolated from kimchi. *Biofactors*, *21*, 123–125.

Hwang, J. W., & Song, Y. O. (2000). The effects of solvent fractions of kimchi on plasma lipid concentration of rabbit-fed high-cholesterol diet. *Journal of the Korean Society of Food Science and Nutrition*, *19*, 204–210.

Islam, M. S., & Choi, H. (2009). Antidiabetic effect of Korean traditional baechu (Chinese cabbage) kimchi in a type 2 diabetes model of rats. *Journal of Medicinal Food*, *12*, 292–297.

Iwasa, M., Aoi, W., Mune, K., Yamauchi, H., Furuta, K., & Sasaki, S. (2013). Fermented milk improves glucose metabolism in exercise-induced muscle damage in young healthy men. *Nutrition Journal*, *16*, 1475–2891. doi: 10.1186. http://dx.doi.org/10.1186/1475-2891-12-83.

Jalali, F., Sharifi, M., & Salehi, R. (2016). Kefir induces apoptosis and inhibits cell proliferation in human acute erythroleukemia. *Medical Oncology*, *33*(1), 7.

Jayabalan, R., Marimuthu, S., Thangaraj, P., Sathishkumar, M., Binupriya, A. R., Swaminathan, K., et al. (2008). Preservation of Kombucha teas effect of temperature on tea components and free radical scavenging properties. *Journal of Agricultural and Food Chemistry*, *56*, 9064–9071.

Jayabalan, R., Baskaran, S., Marimuthu, S., Swaminathan, K., & Yun, S. E. (2010). Effect of Kombucha tea on aflatoxin B1-induced acute hepatotoxicity in albino rats—Prophylactic and curative studies. *Journal of Applied Biological Chemistry*, *53*, 407–416.

Jayabalan, R., Chen, P. N., Hsieh, Y. S., Prabhakaran, K., Pitchai, P., Marimuthu, S., et al. (2011). Effect of solvent fractions of Kombucha tea on viability and invasiveness of cancer cells-characterization of dimethyl 2 (2-hydroxy-2-methoxpropylidine) malonate and vitexin. *Indian Journal of Biotechnology*, *10*, 75.

Jiang, Z. Q., Chen, C., Yang, B., Hebbar, V., & Kong, A. N. T. (2003). Differential responses from seven mammalian cell lines to the treatments of detoxifying enzyme inducers. *Life Sciences*, *72*(20), 2243–2253. https://doi.org/10.1016/S0024-3205(03)00101-2.

Johanningsmeier, S., McFeeters, R. F., Fleming, H. P., & Thompson, R. L. (2007). Effects of *Leuconostoc mesenteroides* starter culture on fermentation of cabbage with reduced salt concentrations. *Journal of Food Science*, *72*(5), M166–M172. https://doi.org/10.1111/j.1750-3841.2007.00372.x.

Kalač, P., Špička, J., Křížek, M., & Pelikánová, T. (2000). The effects of lactic acid bacteria inoculants on biogenic amines formation in sauerkraut. *Food Chemistry*, *70*(3), 355–359. https://doi.org/10.1016/S0308-8146(00)00103-5.

Karabıyıklı, Ş., & Daştan, S. (2016). Microbiologic profile of kefir which is a traditional and functional food. *Journal of Agricultural Faculty of Gaziosmanpasa University*, *33*(1), 75–83.

Karatepe, P., Yalçın, H., & Patır, B. (2012). Kefir and microbiology of kefir. *Electronic Microbiology Journal*, *10*(1), 1–10. (Turkish).

Kharat, M. M., Adiani, V., Variyar, P., Sharma, A., & Singhal, R. S. (2016). Antioxidant compounds in traditional Indian pickles may prevent the process-induced formation of benzene. *Journal of Food Protection*, *79*(1), 123–131. https://doi.org/10.4315/0362-028X.JFP-15-218.

Khasanah, Y. (2013). *Effect of dietary tempe towards the folate nutritional status in Sprague Dawley rats*. Yogyakarta, Indonesia: Universitas Gadjah Mada. Ph.D. thesis, available online http://etd.repository.ugm.Ac.id/index.Phpmod=penelitian_detail&sub=PenelitianDetail&act=view&typ=html&buku_id=63598.

Khasanah, Y., Ratnayani, A. D., Angwar, M., & Nuraeni, T. (2015). In vivo study on albumin and total protein in white rat (*Rattus norvegicus*) after feeding of enteral formula from tempe and local food. *Procedia Food Science*, *3*, 274–279.

Kil, J. H. (2004). *Studies on development of cancer preventive and anticancer kimchi and its anticancer mechanism*. Busan, Korea: Pusan National University. Ph.D. thesis.

Kim, Y. -J. (2007). Antimelanogenic and antioxidant properties of gallic acid. *Biological & Pharmaceutical Bulletin*, *30*, 1052–1055. https://doi.org/10.1248/bpb.30.1052.

Kim, M. J., & Chun, J. S. (2005). Bacterial community structure in kimchi, a Korean fermented vegetable food, as revealed by 16S rRNA gene analysis. *International Journal of Food Microbiology*, *103*, 91–96.

Kim, S. C., Schneeweiss, S., Choudhry, N., Liu, J., Glynn, R. J., & Solomon, D. H. (2015). Effects of xanthine oxidase inhibitors on cardiovascular disease in patients with gout: A cohort study. *The American Journal of Medicine*, *128*, 653.e7–653.e16.

Kitamura, Y., Kusumoto, K. I., Oguma, T., Nagai, T., Furukawa, S., Suzuki, C., et al. (2016). Ethnic fermented foods and alcoholic beverages of Japan. *Ethnic fermented foods and alcoholic beverages of Asia*. India: Springer. pp. 193-236. https://doi.org/10.1007/978-81-322-2800-4_9.

Kök-Taş, T., Ekinci, Y., & Guzel-Seydim, Z. B. (2012). Identification of microbial flora in kefir grains produced in Turkey using PCR. *International Journal of Dairy Technology*, *65*(1), 126–131.

Krajka-Kuźniak, V., Szaefer, H., Bartoszek, A., & Baer-Dubowska, W. (2011). Modulation of rat hepatic and kidney phase II enzymes by cabbage juices: Comparison with the effects of indole-3-carbinol and phenethyl isothiocyanate. *British Journal of Nutrition*, *105*(6), 816–826. https://doi.org/10.1017/S0007114510004526.

Krishnamoorthy, S., Kunjithapatham, S., & Manickam, L. (2013). *Traditional Indian breakfast (idli and dosa) with enhanced nutritional content using millets*. (70). wiley. pp. 241-246. https://doi.org/10.1111/1747-0080.12020.

Kübra, F., Alkan, S. B., & Kubra Sayin, F. (2015). The effect of pickling on total phenolic contents and antioxidant activity of 10 vegetables. *Journal of Food and Health Science Sayın and Alkan*, *1*(3), 135–141. https://doi.org/10.3153/JFHS15013.

Kumar, M., Kumar, A., Nagpal, R., Mohania, D., Behare, P., Verma, V., et al. (2010). Cancer-preventing attributes of probiotics: An update. *International Journal of Food Sciences and Nutrition*, *61*(5), 473–496. https://doi.org/10.3109/09637480903455971.

Kumari, S., Bhinder, S., Singh, B., Kaur, A., & Singh, N. (2020). Effect of buckwheat incorporation on batter fermentation, rheology, phenolic, amino acid composition and textural properties of idli. *LWT—Food Science and Technology*, *122*(January), 109042. https://doi.org/10.1016/j.lwt.2020.109042.

Kusumoto, K. I., & Rai, A. K. (2017). Miso, the traditional fermented soybean paste of Japan. *Fermented foods, Part II: Technological interventions*. (pp. 1–13) CRC Press. Undefined. (n.d.).

Kusznierewicz, B., Lewandowska, J., Kruszyna, A., Piasek, A., Śmiechowska, A., Namieśnik, J., et al. (2010). The antioxidative properties of white cabbage (*Brassica oleracea* var. capitata f. alba) fresh and submitted to culinary processing. *Journal of Food Biochemistry*, *34*(Suppl. 1), 262–285. https://doi.org/10.1111/j.1745-4514.2009.00329.x.

Kwon, M. J., Chun, J. H., Song, Y. S., & Song, Y. O. (1999). Daily kimchi consumption and its hypolipidemic effect in middle-aged men. *Journal of the Korean Society of Food Science and Nutrition*, *28*, 1144–1150.

Lalitha, C. (2016). Sauerkraut – A healthy way to maintain our health. *Journal of Agricultural Engineering and Food Technology*, *3*(2), 70–74.

LeBlanc, J. G., Milani, C., de Giori, G. S., Sesma, F., van Sinderen, D., & Ventura, M. (2013). Bacteria as vitamin suppliers to their host: A gut microbiota perspective. *Current Opinion in Biotechnology*, *24*(2), 160–168. https://doi.org/10.1016/j.copbio.2012.08.005.

Lee, C. W., Ko, C. Y., & Ha, D. M. (1992). Microfloral changes of the lactic acid bacteria during kimchi fermentation and identification of the isolates. *Korean Journal of Applied Microbiology and Biotechnology*, *20*, 102–109.

Lee, S. Y., Song, Y. O., Han, E. S., & Han, J. S. (2012). Comparative study on dietary habits, food intakes, and serum lipid levels according to kimchi consumption in college students. *Journal of the Korean Society of Food Science and Nutrition*, *41*, 351–361.

Lee, M. E., Jang, J. Y., Lee, J. H., Park, H. W., Choi, H. J., & Kim, T. W. (2015). Starter cultures for kimchi fermentation. *Journal of Microbiology and Biotechnology*, *25*(5), 559–568. https://doi.org/10.4014/jmb.1501.01019.

Li, Z., Li, J., Mo, B., Hu, C., Liu, H., Qi, H., et al. (2008). Genistein induces cell apoptosis in MDA- MB-231 breast cancer cells via the mitogen-activated protein kinase pathway. *Toxicology in Vitro*, *22*, 1749–1753.

Libudzisz, Z., & Piatkiewicz, A. (1990). Kefir production in Poland. *Dairy Industries International*, *55*, 31–33.

Liger-Belair, G. (2016). Wines: Champagne and sparkling wines – Production and effervescence. In B. Caballero, P. M. Finglas, & F. Toldrá (Eds.), *Encyclopedia of food and health* (pp. 526–533). Kidlington, Oxford/Waltham, MA: Elsevier Science Ltd. (Academic Press).

Lin, Y., Meijer, G. W., Vermeer, M. A., & Trautwein, E. A. (2004). Soy protein enhances the cholesterol-lowering effect of plant sterol esters in cholesterol-fed hamsters. *Journal of Nutrition*, *134*, 143–148.

Lin, C. H., Wei, Y. T., & Chou, C. C. (2006). Enhanced antioxidative activity of soybean koji prepared with various filamentous fungi. *Food Microbiology*, *23*, 628–633.

Lindow, S. E., & Brandl, M. T. (2003). Microbiology of the phyllosphere. *Applied and Environmental Microbiology*, *69*(4), 1875–1883. https://doi.org/10.1128/AEM.69.4.1875-1883.2003.

Liu, C. H., Hsu, W. H., Lee, F. L., & Liao, C. C. (1996a). The isolation and identification of microbes from a fermented tea beverage, Haipao, and their interactions during Haipao fermentation. *Food Microbiology*, *13*, 407–415.

Liu, C. H., Hsu, W. H., Lee, F. L., & Liao, C. C. (1996b). The isolation and identification of microbes from fermented tea beverage, Haipao, and their interactions during Haipao fermentation. *Food Microbiology*, *13*, 407–415.

Liu, J. R., Chen, M. J., & Lin, C. W. (2002). Characterization of polysaccharide and volatile compounds produced by kefir grains grown in soymilk. *Food and Chemical Toxicology*, *67*, 104–108.

Lorea Baroja, M., Kirjavainen, P. V., Hekmat, S., & Reid, G. (2007). Anti-inflammatory effects of probiotic yogurt in inflammatory bowel disease patients. *Clinical and Experimental Immunology*, *149*, 470–479.

Loretan, T., Mostert, J. F., & Viljoen, B. C. (2003). Microbial flora associated with South African household kefir. *South African Journal of Science*, *99*, 92–94.

Macbean, R. D. (2010). Shelf life of yogurt. *Food Packaging and Shelf Life*, 143–156. https://www.google.com.gh/url?sa=t&rct=j&q=&esrc=s&source=web&cd=3&cad=rja&uact=8&ved=0ahUKEwjjvsTMoJTUAhVoKcAKHViOChcQFggwMAI&url=https%3A%2F%2Fwww.researchgate.net%2Ffile.PostFileLoader.html%3Fid%3D56567db260614b9f538b4567%26assetKey%3DAS%253A2998820080.

Machida, M., Yamada, O., & Gomi, K. (2008). Genomics of *Aspergillus oryzae*: Learning from the history of koji mold and exploration of its future. *DNA Research*, *15*, 173–183. Undefined. (n.d.), Academic.Oup.Com, Retrieved from https://academic.oup.com/dnaresearch/article-abstract/15/4/173/376904 19.06.20.

Malbasa, R., Loncar, E., Vitas, J., & Canadanovic-Brunet, J. M. (2011). Influence of starter cultures on the antioxidant activity of kombucha beverage. *Food Chemistry*, *127*(4), 1727–1731.

Mani, V., & Ming, L. C. (2017). Tempeh and other fermented soybean products rich in isoflavones. *Fermented foods in health and disease prevention* (pp. 453–474). Elsevier Inc.. https://doi.org/10.1016/B978-0-12-802309-9.00019-4.

Marco, M. L., Heeney, D., Binda, S., Cifelli, C. J., Cotter, P. D., Foligné, B., et al. (2017a). Health benefits of fermented foods: Microbiota and beyond. *Current Opinion in Biotechnology*, *44*, 94–102.

Marsh, A. J., O'Sullivan, O., Hill, C., Ross, R. P., & Cotter, P. D. (2013). Sequencing-based analysis of the bacterial and fungal composition of kefir grains and milks from multiple sources. *PLoS One*, *8*, 1–11.

Marsh, A. J., O'Sullivan, O., Hill, C., Ross, R. P., & Cotter, P. D. (2014). Sequence-based analysis of the bacterial and fungal compositions of multiple kombucha (tea fungus) samples. *Food Microbiology*, *38*, 171–178.

Marteau, P., & Rambaud, J. C. (1993). Potential of using lactic bacteria for therapy and immunomodulation in man. *FEMS Microbiology Reviews*, *12*, 207–220.

Martinez-Villaluenga, C., Peñas, E., Sidro, B., Ullate, M., Frias, J., & Vidal-Valverde, C. (2012). White cabbage fermentation improves ascorbigen content, antioxidant and nitric oxide production inhibitory activity in LPS-induced macrophages. *LWT—Food Science and Technology*, *46*(1), 77–83. https://doi.org/10.1016/j.lwt.2011.10.023.

McKinley, M. C. (2005). The nutrition and health benefits of yoghurt. *International Journal of Dairy Technology*, *58*(1), 1–12. https://doi.org/10.1111/j.1471-0307.2005.00180.x.

Messina, M., & Hilakivi-Clarke, L. (2009). Early intake appears to be the key to the proposed protective effects of soy intake against breast cancer. *Nutrition and Cancer*, *61*, 792–798.

Miller, K. C., Mack, G., & Knight, K. L. (2009). Electrolyte and plasma changes after ingestion of pickle juice, water, and a common carbohydrate-electrolyte solution. *Journal of Athletic Training*, *44*(5), 454–461. https://doi.org/10.4085/1062-6050-44.5.454.

Miyamoto, T., Morita, H., Nishioka, K., Kataoka, K., Izumimoto, M., & Kuyama, T. (1991). Constituent species of lactic acid bacteria from kefir and their desmutagenic properties. *Jpn. J. Dairy Federation Sci.*, *40*, 111–112.

Monika, Savitri, Kumar, V., Kumari, A., Angmo, K., & Bhalla, T. C. (2017). Isolation and characterization of lactic acid bacteria from traditional pickles of Himachal Pradesh, India. *Journal of Food Science and Technology*, *54*(7), 1945–1952. https://doi.org/10.1007/s13197-017-2629-1.

Mulmule, M. D., Shimmy, S. M., Bambole, V., Jamdar, S. N., Rawat, K. P., & Sarma, K. S. S. (2017). Combination of electron beam irradiation and thermal treatment to enhance the shelf-life of traditional Indian fermented food (idli). *Radiation Physics and Chemistry*, *131*, 95–99. https://doi.org/10.1016/j.radphyschem.2016.10.014.

Murata, K., Ikehata, H., & Miyamoto, T. (1967). Studies on the nutritional value of tempeh. *Journal of Food Science*, *32*, 580–586.

Murata, K., Miyamoto, T., Kokufu, E., & Sanke, Y. (1970). Studies on the nutritional value of tempeh. 3. Changes in biotin and folic acid contents during tempeh fermentation. *Journal of Vitaminology (Kyoto)*, *16*, 281–284.

Nazni, P., & Shalini, S. (2020). Standardization and quality evaluation of idli prepared from pearl millet (*Pennisetum glaucum*). *International Journal of Current Research*, *5*, 084–087. Retrieved from http://www.journalcra.com 18.06.20.

Noonari, S., & Memon, M. S. I. N. (2015). Performance of pickle production processing and marketing in Sindh Pakistan. *Journal of Food Processing & Technology*, *6*(12), . https://doi.org/10.4172/2157-7110.1000523.

Nout, M. J. R., & Kiers, J. L. (2005). Tempe fermentation, innovation and functionality: Update into the third millennium. *Journal of Applied Microbiology*, *98*, 789–805.

Ogue-Bon, E., Khoo, C., Hoyles, L., McCartney, A. L., Gibson, G. R., & Rastall, R. A. (2011). In vitro fermentation of rice bran combined with *Lactobacillus acidophilus* 14 150B or *Bifidobacterium longum* 05 by the canine faecal microbiota. *FEMS Microbiology Ecology*, *75*, 365–376.

Okouchi, R., Sakanoi, Y., & Tsuduki, T. (2019). Miso (fermented soybean paste) suppresses visceral fat accumulation in mice, especially in combination with exercise. *Nutrients*, *11*, 560. https://doi.org/10.3390/nu11030560.

Oliveira, C. A. F., Telles, E. O., Fernandes, A. M., & Balian, S. (2002). Composition and sensory evaluation of whole yogurt produced from milk with different somatic cell counts. *Australian Journal of Dairy Technology*, *57*, 192–196. October.

Onozawa, M., Fukuda, K., Ohtani, M., Akaza, H., Sugimura, T., & Wakabayashi, K. (1998). Effects of soybean isoflavones on cell growth and apoptosis of the human prostatic cancer cell line LNCaP. *Japanese Journal of Clinical Oncology*, *28*(6), 360–363. https://doi.org/10.1093/jjco/28.6.360.

Otles, S., & Cagindi, O. (2003). Kefir: A probiotic dairy-composition, nutritional and therapeutic aspects. *Pakistan Journal of Nutrition*, *2*(2), 54–59.

Ozer, B. H., & Robinson, R. K. (1999). Rheology and Microstructure of Labneh (Concentrated Yogurt). *Lebensmittel-Wissenschaft und -Technologie*, *32*, 682–689.

Park, K. Y., & Rhee, S. H. (1995). Functional foods from fermented vegetable products: Kimchi (Korean fermented vegetables) and functionality. In J. Shi, C. T. Ho, & F. Shahidi (Eds.), *Asian functional foods* (pp. 341–380). Boca Raton, FL: CRC Press Inc..

Park, K. Y., Baek, K. A., Rhee, S. H., & Cheigh, H. S. (1995). Antimutagenic effect of kimchi. *Food Science and Biotechnology*, *4*, 141–145.

Park, J. A., Pichiah, P. B. T., Yu, J. J., Oh, S. H., Daily, J. W., III, & Cha, Y. S. (2012). Anti-obesity effect of kimchi fermented with *Weissella koreensis* OK1-6 as starter in high-fat diet-induced obese C57BL/6J mice. *Journal of Applied Microbiology*, *113*, 1507–1516.

Parvez, S., Malik, K. A., Ah Kang, S., & Kim, H. Y. (2006). Probiotics and their fermented food products are beneficial for health. *Journal of Applied Microbiology*, *100*(6), 1171–1185.

Pavese, J. M., Krishna, S. N., & Bergan, R. C. (2014). Genistein inhibits human prostate cancer cell detachment, invasion, and metastasis. *American Journal of Clinical Nutrition*, *100*(Suppl. 1), 431S–436S.

Peñas, E., Frias, J., Sidro, B., & Vidal-Valverde, C. (2010a). Chemical evaluation and sensory quality of sauerkrauts obtained by natural and induced fermentations at different NaCl levels from *Brassica oleracea* Var. capitata cv. Bronco grown in Eastern Spain. Effect of storage. *Journal of Agricultural and Food Chemistry*, *58*(6), 3549–3557. https://doi.org/10.1021/jf903739a.

Peñas, E., Frias, J., Sidro, B., & Vidal-Valverde, C. (2010b). Impact of fermentation conditions and refrigerated storage on microbial quality and biogenic amine content of sauerkraut. *Food Chemistry*, *123*(1), 143–150. https://doi.org/10.1016/j.foodchem.2010.04.021.

Peñas, E., Frias, J., Martínez-Villaluenga, C., & Vidal-Valverde, C. (2011). Bioactive compounds, myrosinase activity, and antioxidant capacity of white cabbages grown in different locations of Spain. *Journal of Agricultural and Food Chemistry*, *59*(8), 3772–3779. https://doi.org/10.1021/jf200356m.

Peñas, E., Martinez-Villaluenga, C., Frias, J., Sánchez-Martínez, M. J., Pérez-Corona, M. T., Madrid, Y., et al. (2012). Se improves indole glucosinolate hydrolysis products content, Se-methylselenocysteine content, antioxidant capacity and potential anti-inflammatory properties of sauerkraut. *Food Chemistry*, *132*(2), 907–914. https://doi.org/10.1016/j.foodchem.2011.11.064.

Persson, T., Popescu Bogdan, O., & Cedazo-Minguez, A. (2014). Oxidative stress in Alzheimer's disease: Why did antioxidant therapy fail? *Oxidative Medicine and Cellular Longevity*, *2014*. https://doi.org/10.1155/2014/427318.

Petrušic, M., Radulovic, Z., Paunovic, D., & Obradovic, D. (2011). Isolation and identification of lactic acid bacteria from kombucha tea. In *Conference join event, proceedings* (pp. 180–186).

Pisoschi, A. M., & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. *European Journal of Medicinal Chemistry*, *97*, 55–74. https://doi.org/10.1016/j.ejmech.2015.04.040.

Podsedek, A. (2007). Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. *LWT—Food Science and Technology*, *40*(1), 1–11. https://doi.org/10.1016/j.lwt.2005.07.023.

Raak, C., Ostermann, T., Boehm, K., & Molsberger, F. (2014). Regular consumption of sauerkraut and its effect on human health: A bibliometric analysis. *Global Advances in Health and Medicine*, *3*(6), 12–18. https://doi.org/10.7453/gahmj.2014.038.

Rabie, M. A., Siliha, H., el-Saidy, S., el-Badawy, A. A., & Malcata, F. X. (2011). Reduced biogenic amine contents in sauerkraut via addition of selected lactic acid bacteria. *Food Chemistry*, *129*(4), 1778–1782. https://doi.org/10.1016/j.foodchem.2011.05.106.

Ragy, M. M. (2015). Effect of exposure and withdrawal of 900-MHz-electromagnetic waves on brain, kidney and liver oxidative stress and some biochemical parameters in male rats. *Electromagnetic Biology and Medicine*, *34*(4), 279–284.

Rani, M., Amane, D., & Ananthanarayan, L. (2019). Impact of partial replacement of rice with other selected cereals on idli batter fermentation and idli characteristics. *Journal of Food Science and Technology*, *56*(3), 1192–1201. https://doi.org/10.1007/s13197-019-03582-3.

Reddy, D. C., Rag, M. R., & Reddy, C. R. (1987). *Indian Journal of Dairy Science*, *40*, 393. REDFERN.

Rimada, P. S., & Abraham, A. G. (2006). Kefiran improves rheological properties of glucono-δ-lactone induced skim milk gels. *International Dairy Journal*, *16*, 33–39.

Rosa, D. D., Dias, M. M. S., Grześkowiak, L. M., Reis, S. A., Conceição, L. I., & do Peuzio, M. C. G. (2017). Milk kefir: Nutritional, microbiological and health benefits. *Nutrition Research Reviews*, *30*, 82–96.

Sahoo, S., & Author, C. (2017). Knowledge and awareness about health benefits of indigenous fermented foods: A comprehensive study. *International Journal of Food Science and Nutrition*, *2*(1), 109–113.

Scott, T. L., Rangaswamy, S., Wicker, C. A., & Izumi, T. (2014). Repair of oxidative DNA damage and cancer: Recent progress in DNA base excision repair. *Antioxidants and Redox Signaling*, *20*(4), 708–726. https://doi.org/10.1089/ars.2013.5529.

Sharifi, M., Moridnia, A., & Mortazavi, D. (2017). Kefir: A powerful probiotics with anticancer properties. *Medical Oncology*, *34*, 183.

Sheih, I. C., Fang, T. J., Wu, T. K., & Chen, R. Y. (2014). Effects of fermentation on antioxidant properties and phytochemical composition of soy germ. *Journal of the Science of Food and Agriculture*, *94*, 3163–3170.

Shinde, A. N., Malpathak, N., & Fulzele, D. P. (2010). Determination of isoflavone content and antioxidant activity in *Psoralea corylifolia* L. callus cultures. *Food Chemistry*, *118*, 128–132.

Sievers, M., Lanini, C., Weber, A., Schuler-Schmid, U., & Teuber, M. (1995). Microbiology and fermentation balance in a kombucha beverage obtained from a tea fungus fermentation. *Systematic and Applied Microbiology*, *18*, 590–594.

Simova, E., Beshkova, D. M., Angelov, A., Hristozova, T., Frengova, G., & Spasov, Z. (2002). Lactic acid bacteria and yeasts in kefir grains and kefir made from them. *Journal of Industrial Microbiology & Biotechnology*, *28*, 1–6.

Soka, S., Suwanto, A., Sajuthi, D., & Rusmana, I. (2014). Impact of tempeh supplementation on gut microbiota composition in Sprague-Dawley rats. *Research Journal of Microbiology*, *9*, 189–198.

Song, A. A., In, L. L. A., Lim, S. H. E., & Rahim, R. A. (2017). A review on *Lactococcus lactis*: From food to factory. *Microbial Cell Factories*, *16*, 55.

Sreeramulu, G., Zhu, Y., & Knol, W. (2000). Kombucha fermentation and its antimicrobial activity. *Journal of Agricultural and Food Chemistry*, *48*, 2589–2594.

Sreeramulu, G., Zhu, Y., & Knol, W. (2001). Characterization of antimicrobial activity in Kombucha fermentation. *Acta Biotechnologica*, *21*, 49–56.

Starzynska-Janiszewska, A., Stodolak, B., & Mickowska, B. (2014). Effect of controlled lactic acid fermentation on selected bioactive and nutritional parameters of tempeh obtained from unhulled common bean (*Phaseolus vulgaris*) seeds. *Journal of the Science of Food and Agriculture*, *94*, 359–366.

Suzuki, R., Kang, Y., Li, X., Roife, D., Zhang, R., & Fleming, J. B. (2014). Genistein potentiates the antitumor effect of 5-fluorouracil by inducing apoptosis and autophagy in human pancreatic cancer cells. *Anticancer Research*, *34*, 4685–4692.

Svensson, M., Sabharwal, H., Mossberg, A. K., Lipniunas, P., Leffler, H., Svanborg, C., & Linse, S. (1999). Molecular characterization of alphalactalbumin folding variants that induce apoptosis in tumor cells. *J. Biol. Chem.*, *274*, 6388–6396.

Swain, M. R., Anandharaj, M., Ray, R. C., & Rani, R. P. (2014). Fermented fruits and vegetables of Asia: A potential source of probiotics. *Biotechnology Research International*, *2014*, 250424. http://dx.doi.org/10.1155/2014/250424.

Szaefer, H., Krajka-Kuźniak, V., Bartoszek, A., & Baer-Dubowska, W. (2012). Modulation of carcinogen metabolizing cytochromes P450 in rat liver and kidney by cabbage and sauerkraut juices: Comparison with the effects of indole-3-carbinol and phenethyl isothiocyanate. *Phytotherapy Research*, *26*(8), 1148–1155. https://doi.org/10.1002/ptr.3692.

Tamang, J. P. (2012). Plant-based fermented foods and beverages of Asia. In Y. H. Hui, & O. Evranuz (Eds.), *Handbook of plant-based fermented food and beverage technology* (pp. 49–90). Boca Raton, FL, USA: CRC Press.

Tamime, A. Y., & Deeth, H. C. (1980). Yogurt: Technology and biochemistry. *Journal of Food Protection*, *43*(12), 939–977. https://doi.org/10.4315/0362-028x-43.12.939.

Tamime, A. Y., & Robinson, R. K. (1994). *Tamime Robinson's yoghurt* (3rd ed., pp. 1–808). elsevier. Retrieved from https://www.elsevier.com/books/tamime-and-robinsons-yoghurt/tamime/978-1-84569-213-1 24.06.20.

Tamime, A. Y., Saarela, M., Korslund-Søndergaard, A., Mistry, V. V., & Shah, N. P. (2005). Production and maintenance of viability of probiotic micro-organisms in dairy products. In A. Y. Tamime (Ed.), *Probiotic dairy products* (pp. P44–P51). Oxford, UK: Blackwell Publishing.

Tapsell, L. C. (2015). Fermented dairy food and CVD risk. *British Journal of Nutrition*, *2015*(113), 131–135.

Tatsuya, M., Erika, Y., Yuusuke, S., Kyoko, N., Nobuhiro, Z., & Yukio, K. (2013). Hypoallergenicity of various miso pastes manufactured in Japan. *J Nutr Sci Vitaminol (Tokyo)*, pp. 462–469.

Taylor, C. K., Levy, R. M., Elliott, J. C., & Burnett, B. P. (2009). The effect of genistein aglycone on cancer and cancer risk: A review of in vitro, preclinical, and clinical studies. *Nutrition Reviews*, *67*, 398–415.

Teoh, A. L., Heard, G., & Cox, J. (2004). Yeasts ecology of kombucha fermentation. *International Journal of Food Microbiology*, *95*, 119–126. https://doi.org/10.1016/j.ijfoodmicro.2003.12.020.

Titus, A. D., Shankaranarayana Rao, B. S., Harsha, H. N., Ramkumar, K., Srikumar, B. N., Singh, S. B., et al. (2007). Hypobaric hypoxia-induced dendritic atrophy of hippocampal neurons is associated with cognitive impairment in adult rats. *Neuroscience*, *145*, 265–278.

Tolonen, M., Taipale, M., Viander, B., Pihlava, J. M., Korhonen, H., & Ryhänen, E. L. (2002). Plant-derived biomolecules in fermented cabbage. *Journal of Agricultural and Food Chemistry*, *50*(23), 6798–6803. https://doi.org/10.1021/jf0109017.

Tolonen, M., Rajaniemi, S., Pihlava, J. -M., Johansson, T., Saris, P. E., & Ryhänen, E. -L. (2004). Formation of nisin, plant-derived biomolecules and antimicrobial activity in starter culture fermentations of sauerkraut. *Food Microbiology*, *21*(2), 167–179. https://doi.org/10.1016/S0740-0020(03)00058-3.

Tse, G., & Eslick, G. D. (2014). Cruciferous vegetables and risk of colorectal neoplasms: A systematic review and meta-analysis. *Nutrition and Cancer*, *66*(1), 128–139. https://doi.org/10.1080/01635581.2014.852686.

Turan, I., & Ilter, T. (2007). Kefir, from the Caucasus Mountains to present day. *Current Gastroenterology*, *11*(2), 65–75. (Turkish).

Ueland, T., Gullestad, L., Nymo, S. H., Yndestad, A., Aukrust, P., & Askevold, E. T. (2015). Inflammatory cytokines as biomarkers in heart failure. *Clinica Chimica Acta*, *443*, 71–77. https://doi.org/10.1016/j.cca.2014.09.001.

Uraz, T. (2014). Molds employed in food processing. *Encyclopedia of Food Microbiology*, *3*, 522–528. https://doi.org/10.1016/B978-0-12-384730-0.00323-2.

Vaidehi, M. P., Sumangala, S. G., & Vijayakumari, J. (1996). Tempe-based ready-to-prepare food mixes of high nutritional value. *Journal of Food Science and Technology*, *33*, 506–509.

Velicanski, A. S., Cvetkovic, D. D., Markov, S. L., Tumbas Šaponjac, V. T., & Vulic´, J. J. (2014). Antioxidant and antibacterial activity of the beverage obtained by fermentation of sweetened lemon balm (*Melissa officinalis* L.) tea with symbiotic consortium of bacteria and yeasts. *Food Technology and Biotechnology*, *52*(4), 420–429.

Verkerk, R., Schreiner, M., Krumbein, A., Ciska, E., Holst, B., Rowland, I., et al. (2009). Glucosinolates in Brassica vegetables: The influence of the food supply chain on intake, bioavailability and human health. *Molecular Nutrition and Food Research*, *53*(Suppl. 2), 219–265. https://doi.org/10.1002/mnfr.200800065.

Wacher, C., Díaz-Ruiz, G., & Tamang, J. (2010). Fermented vegetable products. Fermented foods and beverages of the world, July, 149-190. https://doi.org/10.1201/ebk1420094954-c5.

Walsh, A. M., Crispie, F., Kilcawley, K., O'Sullivan, O., O'Sullivan, M. G., Claesson, J. J., et al. (2016). Microbial succession and flavor production in the fermented dairy beverage kefir. *mSystems*, *1*, 1–16.

Wang, J. -G., Chen, X., Liu, W. -J., Yang, M., Airidengcaicike, & Zhang, H. -P. (2008). Identification of *Lactobacillus* from koumiss by conventional and molecular methods. *European Food Research and Technology*, *227*(5), 1555–1561.

Wang, Y., Xu, N., Xi, A., et al. (2009). Effects of *Lactobacillus plantarum* MA2 isolated from Tibet kefir on lipid metabolism and intestinal microflora of rats fed on high-cholesterol diet. *Applied Microbiology and Biotechnology*, *84*, 341–347.

Wang, S., Tamura, T., Kyouno, N., Liu, X., Zhang, H., & Akiyama, Y. (2019). Effect of the chemical composition of miso (Japanese fermented soybean paste) upon the sensory evaluation. *Analytical Letters*, *52*(11), 1813–1827. https://doi.org/10.1080/00032719.2019.1570244.

Watawana, M. I., Jayawardena, N., Gunawardhana, C. B., & Waisundara, V. Y. (2015). Health, wellness, and safety aspects of the consumption of kombucha. *Journal of Chemotherapy*, *2015*, 1–11.

Wiander, B., & Ryhänen, E. L. (2005a). Laboratory and large-scale fermentation of white cabbage into sauerkraut and sauerkraut juice by using starters in combination with mineral salt with a low NaCl content. *European Food Research and Technology*, *220*(2), 191–195. https://doi.org/10.1007/s00217-004-1080-5.

Wiander, B., & Ryhänen, E. -L. (2005b). Laboratory and large-scale fermentation of white cabbage into sauerkraut and sauerkraut juice by using starters in combination with mineral salt with a low NaCl content. *European Food Research and Technology*, *220*(2), 191–195. https://doi.org/10.1007/s00217-004-1080-5.

Witthuhn, R. C., Schoeman, T., & Britz, T. J. (2005). Characterisation of the microbial population at different stages of kefir production and kefir grains mass cultivation. *International Dairy Journal*, *15*, 383–389.

Wolkers-Rooijackers, J. C. M., Thomas, S. M., & Nout, M. J. R. (2013). Effects of sodium reduction scenarios on fermentation and quality of sauerkraut. *LWT—Food Science and Technology*, *54*(2), 383–388. https://doi.org/10.1016/j.lwt.2013.07.002.

Xu, D. P., Xia, G., Hh, J., & Ck, Z. (2002). Effect of tempe isoflavones on tumor. *Journal of Nanjing Agricultural University*, *25*, 97–101.

Yadav, H., Jain, S., & Sinha, P. R. (2007). Antidiabetic effect of probiotic dahi containing *Lactobacillus acidophilus* and *Lactobacillus casei* in high fructose fed rats. *Nutrition*, *23*(1), 62–68. https://doi.org/10.1016/j.nut.2006.09.002.

Yang, Z. W., Ji, B. P., Zhou, F., Li, B., Luo, Y., Yang, L., et al. (2009). Hypocholesterolaemic and antioxidant effects of kombucha tea in high-cholesterol fed mice. *Journal of the Science of Food and Agriculture*, *89*, 150–157.

Younus, S., Masud, T., & Aziz, T. (2002). Quality evaluation of market yoghurt/dahi. *Pakistan Journal of Nutrition*, *1*(5), 226–230.

Zeng, X. Q., Pan, D. D., & Guo, Y. X. (2010). The probiotic properties of *Lactobacillus buchneri* P2. *Journal of Applied Microbiology*, *108*, 2059–2066.

Zubillaga, M., Weill, M. R., & Postaire, E. (2001). Effect of probiotics and functional foods and their uses in different diseases. *Nutrition Research*, *21*, 569–579.

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Chapter 24

Lactic Acid Bacteria in Fermented Food

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1 Introduction

Fermented foods are popular foods around the world, and the vast majority of traditional fermented foods may be classified as probiotic foods in the future (Attri, Khaket, Jodha, Singh, & Dhanda, 2015). Fermentation is generally defined as the metabolic process that takes energy from organic compounds without involving foreign oxidants (Ray & Joshi, 2014). For centuries, diverse plant and animal materials have been fermented by various bacteria, yeasts, and fungi to make excellent foods (Hesseltine & Wang, 1967). Food fermentation is regarded as one of the oldest ways of food processing and preservation (Achi, 2005). Probiotics microorganisms are utilized when manufacturing food by fermentation. The most commonly used are lactic acid bacteria, acetic acid bacteria, yellow bacillus, coryneform bacteria, and others like in yeast, *Aspergillus*, and bacteria.

Fermentation can increase the shelf life of foods such as meat, fish, fruits, and vegetables. Finished foods typically have improved microbial stability and safety, and these foods can generally be stored at ambient temperature for extended periods of time. In addition to allowing food to be stored for longer periods of time, fermentation can also be used to develop a wider variety of flavors, aromas, and tastes to alter the sensory characteristics of the food. In addition, fermentation can increase digestibility and nutritional quality through a food matrix rich in vitamins, proteins, essential amino acids, and fatty acids, and more specifically, the mobility of fermenting microbial communities and their properties to the energetics of the process product quality.

Bacteria, yeast, and mold are the more common microbial groups in food fermentation. Among the bacteria in fermented foods, *Lactobacillus* plays a pivotal role and its main function is to convert carbohydrates into lactic acid. In addition, *Acetobacter* (mainly from the fermentation of fruits and vegetables) and *Bacillus* (fermentation from legumes) are also widely used in the fermentation of foods. Yeasts are widely used in the food industry such as the production of wine, beer and ethanol, and leavening of bread because of the efficient action of the enzymes they produce.

The foods produced by these microbial activities usually have the following five categories (representative fermented foods and microorganisms used therein): alcoholic beverages such as distilled liquor, rice wine, fruit wine, and beer; dairy products such as yogurt, sour cream, kumiss, and cheese; soy products such as bean curd, cardamom, and natto; fermented vegetables such as kimchi, and sauerkraut; condiments such as vinegar, yellow sauce, and soy sauce; sweeteners such as monosodium glutamate (MSG); and flavor enhancers (such as 5′-nucleotides) and MSG.

If the food is to be fermented, it must have a specific microbial environment. The type of microorganisms in the fermentation process will have a great influence on the taste of the fermented food, and strengthening the research on the microbial diversity in the fermented food can be very effective for related fields. The study provides more theoretical basis. In the process of research, the two most basic elements are the abundance of species and the uniformity of species. Microorganisms also have their own unique characteristics in the process of growth. Because the microbial size itself is small, the structure is not very complicated, but it is still in a relatively slow state in the study of microbial diversity. For a long time, people have used very old methods to study the diversity of microorganisms. With the continuous development of related technologies, there are some new signs in microbiology research.

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00024-1
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Microorganisms further decompose the original protein and sugar of food into amino acids, lactic acid, and other substances. The overall structure changes, the taste is naturally different, and newly produced amino acids and other substances also add special flavor to the food. Moreover, their masterpieces are always as mysterious as artistic creation. With the development of science and technology, although we have been able to intervene and control certain aspects of fermentation, we can also use special strains or yeast cultivated artificially to achieve specific fermentation effects. However, microbial activity is still more wonderful, complex and elegant than we think. We still cannot make two bottles of the same wine, even with the same site, tools, raw materials, strains, yeast, temperature, humidity, time, manpower, etc. In the future, probiotics microorganisms will be applied to a wider space such as health care, flavor food, and food industry, bringing more rich and beautiful life quality to everyone.

2 The probiotics microorganism used in the fermented food

The bacteria used in the fermented food mainly include three kinds of bacteria: *Acetobacter*, nonpathogenic *Corynebacterium*, and lactic acid bacteria.

2.1 Acetobacter

Acetobacter is commonly found in rotten fruits, vegetables, sour juices, vinegar, and beverages. It is a Gram-negative non-*Bacillus*, facultative aerobic but prone to degeneration. Degenerate bacteria show a curved shape such as dendritic or filamentous. Gram staining of strains in old cultures also often changes. *Acetobacter* can oxidize ethanol to make it acetic acid and is therefore the main species for the manufacture of vinegar. Vinegar is a traditional condiment in China's major cuisines. According to the existing written records, the ancient Chinese working people used wine as a starter to ferment and produce vinegar. Oriental vinegar originated in China. According to the literature, the history of vinegar is at least 3000 years. *Acetobacter* is a type of *Brevibacterium* that oxidizes sugars and alcohols to products such as acetic acid. *Acetobacter* has no spores, cannot move, has both aerobic and anaerobic strains, and easily forms a membrane on the surface of liquid medium. In the industry, acetic acid can be used to make vinegar, acetic acid, and gluconic acid.

2.2 Nonpathogenic *Corynebacterium*

Nonpathogenic *Corynebacterium* is often isolated from soil, water, air, and contaminated bacterial culture dishes or blood plates. *Corynebacterium glutamicum*, *Corynebacterium faecalis*, and *Corynebacterium aureus* in nonpathogenic *Corynebacterium* are often used for the production of MSG (L-glutamate). They can decompose sugar into organic acids, decompose nitrogen-containing substances into ammonium ions, and further synthesize glutamic acid and accumulate in the fermentation broth. MSG has a strong umami taste (diluted 300 times still has an umami taste) and is sodium L-glutamate containing one molecular crystal water. MSG quickly decomposes glutamate into the human body, so the physiological action of sodium glutamate is the same as that of glutamic acid. Glutamate is a normal metabolite in the human body. It has an important role in human metabolism, such as the human body needs protein synthesis and metabolism of the brain involved in protein and carbohydrates, as well as promotes the oxidation process. It is not only a part of the brain tissue metabolism but also a brain. Acid cells may be utilized. According to foreign reports, glutamic acid can rapidly improve the mental retardation of children's intelligence. It affects neural activity produced by acetylcholine.

2.3 Lactic acid bacteria

Lactic acid bacteria produce lactic acid, which is a type of bacteria that plays a major role in the production of fermented dairy products. According to its characteristics of sugar fermentation, it can be divided into homofermentative bacteria and heterofermentation bacteria. Any bacterium that produces lactic acid from the fermentation of glucose or lactose is collectively referred to as lactic acid bacteria. This is a group of quite complex bacteria, currently divided into at least 18 genera, a total of more than 200. Except for a very small number, most of them are indispensable and important physiological functions in the human body, which are widely present in the intestines of the human body. It has been confirmed by biologists at home and abroad that intestinal lactic acid bacteria have a very close relationship with health and longevity.

In the fermentation process, the same type of fermenting bacteria can convert 80%–90% of the lactose in the fermentation broth into lactic acid with only a small number of other by-products. Commonly used strains are *Lactobacillus casei*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus helveticus*, *Lactobacillus lactis*, *Streptococcus lactis*, *Streptococcus thermophilus*, and new subspecies of *Streptococcus faecalis*.

In the fermentation process, the heterologous fermenting bacteria can convert 50% of the lactose into lactic acid in the fermentation broth, and the other 50% of the sugar is converted into other organic acids, alcohols, carbon dioxide, hydrogen, and the like. The strains used in foods are *Leuconostoc cerevisiae* and *L. lactis*.

Fermented lactic acid bacteria food not only has a good taste but also has many benefits to the human body:

1. It can prevent lactose intolerance (swelling, diarrhea, and other symptoms that occur when drinking fresh milk).
2. It promotes the absorption of nutrients such as proteins, monosaccharides, and calcium and magnesium and produces a large number of beneficial substances such as vitamin B.
3. Increases the beneficial bacteria in the intestines and improves the function of the human gastrointestinal tract, restores the balance of the flora in the human intestinal tract, forms an antibacterial biological barrier, and maintains human health.
4. Inhibits the reproduction of spoilage bacteria, eliminates the toxins produced by spoilage bacteria, and removes intestinal waste.
5. Inhibition of cholesterol absorption, lowering blood fat, and lowering blood pressure.
6. Immune regulation enhances human immunity and resistance.
7. Antitumor, prevent cancer.
8. Improves the activity of Superoxide Dismutase (SOD), eliminates free radicals in the human body, and has antiaging and longevity effects.
9. Effectively prevents bacterial infection of the female genitourinary system.
10. Controls the level of toxins in the body, protects the liver, and enhances the detoxification and detoxification functions of the liver.

A small number of heterofermental fermentations, such as *Leuconostoc mesogenes*, are important strains for the production of dextran (i.e., plasma) in the pharmaceutical industry but are also pests of the sugar industry, often making the juice thick and incapable of processing. *Lactobacillus* family, the rods are rod-shaped, single or chained, sometimes filamentous, producing false branches. According to the different products after using glucose, it is divided into homofermentation group and heterotypic fermentation group. Most of the fermentable lactose, without the use of lactic acid, can lower the pH to below 6.0 after fermentation.

Among the families, *Lactobacillus* is the most important, and most of them are commonly used in different industries, especially in the food industry. It is found in dairy products and fermented plant foods such as kimchi, sauerkraut, silage, and human intestines, especially in the intestines of infants. Industrial production of lactic acid commonly used in high temperature fermentation bacteria. For example, *Lactobacillus delbrueckii* has an optimum growth temperature of 45°C and is widely used in the manufacture of lactic acid and calcium lactate manufacturing industries (Table 24.1).

2.4 Yeast

Yeast fungi (Oelschlaeger, 2010) cells are mostly single cells, spherical, oval, cylindrical, lemon-shaped, pear-shaped, and the like. Under certain conditions, certain strains form elongated long-chain cells, which are similar in shape to mycelial hyphae and are called pseudohyphae. The size of the yeast cells varies depending on the medium components and the age of the bacteria, and is generally (8–10) × (1–5) μm.

Yeast cells contain proteins, carbohydrates, fats, vitamins, enzymes, and inorganic salts. The protein content (on a dry basis) is generally 51%–55% and some even higher. There are more than 13 kinds of amino acids constituting this protein, which have high nutritional value and are easy to digest and absorb. Vitamins are also abundant, and there are more than 14 known, and most of them are water-soluble. Therefore, yeast is a good protein resource.

The fermentation of yeast cells or yeast can produce a variety of foods such as alcohol, taro, bread, and single cell protein.

Excessive nucleic acid can cause diseases such as gout and kidney stones in the human body. Therefore, the yeast for consumption must be refined to remove nucleic acids. Yeast extracts can be used for flavoring, tonics, and fillers in bionutrition and nutritious foods.

The yeasts commonly used in the food industry are *Saccharomyces cerevisiae*, elliptic yeast, Karl yeast, and *Hansenula*.

S. cerevisiae are mostly elliptical with a ratio of length to width of 2:1. They are greater endurance to alcohol, can ferment glucose, maltose, galactose, sucrose, and one-third raffinose, cannot ferment lactose and melibiose, and cannot assimilate nitrate. It is often found in koji, peel, fermented juice, and soil in orchards. It is the most commonly used bacteria in the wine industry and is also used in beer brewing; it can also ferment bread; its invertase can be used to transform sugar and can also be used in the production of chocolate.

TABLE 24.1 Lactic acid bacteria fermented food.

Food	Country	Ingredients	Microorganisms	Health/Nutrition benefits	References
Acidophilus milk	Various countries	Cow milk	*Lacidophilus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus paracasei* subsp. *paracasei*, *L. paracasei* subsp. *pseudoplantarum*	Intestinal disorders including chronic constipation, diarrhea, colitis, sprue	Caplice and Fitzgerald (1999), Steinkraus (1983), Marteau, Flourie, and Pochart (1990)
Amasi or mukaka wakora or zifa	Zimbabwe	Milk	*Lactobacillus lactis* subsp. *lactis*, *L. lactis* subsp. *lactis biovar diacetylactis*, *L. paracasei* subsp. *paracasei*, *Lactobacillus plantarum*, *L. acidophilus*, *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Enterococcus faecium*, *Enterococcus faecalis*	Regulate intestinal flora balance, prevent pathogenic bacteria in the antibody, prevent diarrhea, enhance immunity	Gadaga, Mutukumira, Narvhus, and Feresu (1999)
Ayib	Ethiopia	Goat milk	*Lactobacillus* sp., *Lactococcus* sp.	Healthy human gastrointestinal tract	Oyewole (1997)
Butter milk (various local names)	Various countries	Cow milk	*Lactobacillus bulgaricus*, *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis biovar diacetylactis*, *L. mesenteroides* subsp. *Cremoris*	Regulate intestinal flora balance, prevent pathogenic bacteria in the antibody, prevent diarrhea, enhance immunity	Steinkraus (1983), Tamime & Marshall, (1997)
Caplis	Japan	Cow milk	*L. helveticus*, *Saccharomyces cerevisiae*	Produce folic acid and supplement folic acid	Tamime & Marshall, (1997)
Cheese (various local names)	Various countries	Cow milk, or goat milk, or sheep milk	*L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *Streptococcus thermophilus*, *L. helveticus*, *L. delbrueckii* subsp. *bulgaricus*, *yeasts*, *molds*	Intestinal disorders including chronic constipation, diarrhea, colitis, sprue	Caplice and Fitzgerald (1999)
Dahi (Indian yogurt)	India	Cow milk or buffalo milk	*L. lactis* subsp. *cremoris*, *1 L. lactis* subsp. *lactis*, *2 S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus 6*, *L. acidophilus*, *L. helveticus*, *Lactobacillus fermentum*, *Lactobacillus casei*, *L. plantarum*	Intestinal disorders (dyspepsia, dysentery, diarrhea, etc.); improved digestion, antibacterial effects in gut	Abdel Gadir, Mohamed, Abd-el-Malek, Ahmad, & Arroyo, (1983)
Ergo or irgo	Ethiopia	Cow milk	*Lactobacillus* sp., *Streptococcus* sp.	Degradation of fat, protein, so that yogurt is more conducive to digestion and absorption	Tamime & Marshall, (1997)
Iria ri matii	Kenya	Cow milk	*S. thermophilus*	Helps people with lactose intolerance to digest lactose	Tamime & Marshall, (1997)
Ititu	Ethiopia	Cow milk		Increased nutritional quality	Tamime & Marshall, (1997)
Jben	Morocco	Sheep milk		Increased nutritional quality	Oyewole (1997)

TABLE 24.1 Lactic acid bacteria fermented food. (*Cont.*)

Food	Country	Ingredients	Microorganisms	Health/Nutrition benefits	References
Katyk	Kazakhstan	Cow milk	*S. thermophilus*, *L. lactis* subsp. *lactis biovar diacetylactis*, *3 L. helveticus*, *L. delbruecki* subsp. *bulgaricus*	Intestinal disorders including chronic constipation, diarrhea, colitis, sprue	Tamime & Marshall, (1997)
Kefir	Russia	Cow milk or goat milk or sheep milk	*Lactobacillus* sp., *Leuconostoc* sp., *Lactococcus* sp. *yeasts*	Antitumor properties; digestive system disorders	Steinkraus (1983), Farnworth (2003)
Kehran or karan or heran or lapte-akru	Siberia, Rumania	Cow milk	*S. thermophilus*	It helps people with lactose intolerance to digest lactose	Tamime & Marshall, (1997)
Kishk or kisk, or kushuk (various local names)	Egypt, Syria, Lebanon (many other countries)	Milk (yogurt) and wheat	*L. casei*, *L. plantarum*, *Lactobacillus brevis*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus megaterium*, *yeasts*	Increased nutritional quality	Blandino, Al-Aseeri, and Pandiella (2003), Steinkraus (1983)
Koumiss or kumys or coomys	Russia	Mare milk, or cow milk	*L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus*, *Torula yeast*	Treatment of pulmonary tuberculosis	Steinkraus (1983), Tamime & Marshall, (1997)
Liban	Lebanon	Cow, sheep, goat, or camel milk	*S. thermophilus*, *L. lactis*, *L. acidophilus*, *Kluyveromyces fragilis*, *S. cerevisiae*	It helps people with lactose intolerance to digest lactose	Baroudi and Collins (1976)
Laban rayeb or leban rayeb, or raib	Egypt	Cow or sheep milk	*L. lactis* subsp. *lactis*, *S. kefir*, *L. casei*, *Streptococcus faecalis*		Abdel Gadir, Mohamed, Abd-el-Malek, Ahmad, & Arroyo, (1983), Oyewole (1997)
Leban zeer	Egypt	Cow milk	*L. casei*, *L. plantarum*, *L. brevis*	Reduce serum cholesterol levels and prevent cardiovascular disease; maintain intestinal flora balance; promote nutrient absorption; relieve lactose intolerance; inhibit tumor cell formation	Abdel Gadir, Mohamed, Abd-el-Malek, Ahmad, & Arroyo, (1983)
Leben (or lben)	Zimbabwe, Morocco	Cow milk	*Lactic acid bacteria*, *L. lactic* subsp. *lactis*, *L. lactis* subsp. *lactis biovar diacetylactis*, *3 L. lactis*, *L. mesenteroides* subsp. *cremoris*, *L. mesenteroides* subsp. *dextranicum*, *Lactobacillus* sp., *yeasts*, *molds*	Reduce serum cholesterol levels and prevent cardiovascular disease	Oyewole (1997), Baroudi and Collins (1976), Steinkraus (1983)
Liban	Iraq	Cow milk		Increased nutritional quality	Abdel Gadir, Mohamed, Abd-el-Malek, Ahmad, & Arroyo, (1983), Oyewole (1997)
Lyubitelskii	USSR	Cow milk	*S. thermophilus*, *L. lactis* subsp. *lactis biovar diacetylactis*	Help people with lactose intolerance to digest lactose	Tamime & Marshall, (1997)

(*Continued*)

TABLE 24.1 Lactic acid bacteria fermented food. (*Cont.*)

Food	Country	Ingredients	Microorganisms	Health/Nutrition benefits	References
Miziwa lala or mala	Kenya	Cow milk	*L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis biovar diacetylactis*, *3 L. mesenteroides* subsp. *cremoris*	Maintain intestinal flora balance	Tamime & Marshall, (1997)
Nono	West Africa	Cow, sheep milk	*L. acidophilus*, *L. lactis* subsp. *cremoris*, *4 L. delbrueckii* subsp. *bulgaricus*, *L. helveticus*, *L. plantarum*, *L. lactis* subsp. *cremoris*	Reduce serum cholesterol levels and prevent cardiovascular disease; maintain intestinal flora balance; promote nutrient absorption; relieve lactose intolerance; inhibit tumor cell formation	Oyewole (1997), Tamime & Marshall, (1997)
Nordic or Scandinavian sour milk (various local names)	Norway, Sweden	Cow milk	*Lactic acid bacteria*, *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis biovar diacetylactis*, *3 L. mesenteroides* subsp. *cremoris*	Maintain intestinal flora balance	Tamime & Marshall, (1997)
Skyr	Iceland	Cow milk	*S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. helveticus*	Help people with lactose intolerance to digest lactose	Tamime & Marshall, (1997)
Sour milk	Egypt	Cow milk	*L. lactis* subsp. *lactis*, *2 S. kefi*, *5 S. cittrovorus*, *L. casei*, *L. plantarum*, *L. brevis*, *micrococci*, *coliforms*	Reduce serum cholesterol levels and prevent cardiovascular disease; maintain intestinal flora balance; promote nutrient absorption; relieve lactose intolerance; inhibit tumor cell formation	Abdel Gadir, Mohamed, Abd-el-Malek, Ahmad, & Arroyo, (1983)
Susa	Kenya	Camel milk			Tamime & Marshall, (1997)
Suusaac	Somalia	Camel milk		Increased nutritional quality	Tamime & Marshall, (1997)
Tairu	Malaysia	Cow milk or soybean milk		Increased nutritional quality	Abdel Gadir, Mohamed, Abd-el-Malek, Ahmad, & Arroyo, (1983)
Trahanas or tarhanas	Greece, Turkey	Wheat, sheep milk	*Lactic acid bacteria*	Promote nutrient absorption; relieve lactose intolerance	
Viili	Finland	Cow milk	*L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis biovar diacetylactis*, *L. mesenteroides* subsp. *cremoris*, *Geotrichum candidum*	Reduce serum cholesterol levels and prevent cardiovascular disease; maintain intestinal flora balance; promote nutrient absorption; relieve lactose intolerance; inhibit tumor cell formation	Tamime & Marshall, (1997)

TABLE 24.1 Lactic acid bacteria fermented food. (*Cont.*)

Food	Country	Ingredients	Microorganisms	Health/Nutrition benefits	References
Ymer	Denmark	Cow milk	*L. lactis* subsp. *lactis biovar diacetylactis*, *3 L. mesenteroides* subsp. *cremoris*	Reduce serum cholesterol levels and prevent cardiovascular disease; maintain intestinal flora balance; promote nutrient absorption; relieve lactose intolerance; inhibit tumor cell formation	Tamime & Marshall, (1997)
Yakult	Japan	Cow milk	*L. paracasei* subsp. *paracasei*		Tamime & Marshall, (1997)
Yogurt (also known as dahi, leben, goidu, dahi)	Various countries	Cow milk, primarily, sheep milk, goat milk, buffalo milk	*L. bulgaricus*, *L. acidophilus*, *L. casei*, *S. thermophilus*, *L. lactis* subsp. *lactis 2*	Reduce serum cholesterol levels and prevent cardiovascular disease; maintain intestinal flora balance; promote nutrient absorption; relieve lactose intolerance; inhibit tumor cell formation	Tamime & Marshall, (1997)
Zabadi (yogurt)	East, North Africa	Goat, cow, or sheep milk	*L. bulgaricus*, *L. acidophilus*, *L. lactis* subsp. *cremoris*	Promote nutrient absorption; relieve lactose intolerance	Tamime & Marshall, (1997)
Žinčica	Žinčica (in Slovakia), Žinčice (in the Czech Republic), Żentyca (in Poland)	Sheep milk	*L. casei*, *L. plantarum*, *Lactococcus lactis*, and *L. mesenteroides*	Excellent resistance to gastrointestinal digestive fluids. It regulates blood lipids, protects, and repairs liver from hyperlipidemia, and enhances the body's antioxidant capacity	Oyewole (1997)

Elliptic yeast cells are oval, and their other biochemical characteristics are like those of *S. cerevisiae*. In addition to being resistant to higher concentrations of ethanol, they are also resistant to higher grape juice acidity and lower concentrations of sulfur dioxide and are therefore commonly used in winemaking.

Carl yeast is a typical bottom yeast in beer brewing. Its morphology and biochemical properties are like those of *S. cerevisiae*, except that it has the ability to fully ferment raffinose.

Abnormal *Hansenula* cells are round, oval, or sausage-shaped. Developed pseudohyphae can be produced under certain conditions. It can ferment glucose, sucrose, maltose, galactose, and raffinose; it cannot ferment melibiose and lactose. Since it can produce ethyl acetate, it can play a role in improving the flavor of the food. Such bacteria can be used for the aroma of white wine and salt-free fermented soy sauce.

2.4.1 Making noodles

There are many ways to make hair such as baking soda and yeast noodles.

The effect of these methods is the same, that is, by generating a large amount of carbon dioxide gas in the dough, the carbon dioxide is heated and expanded during the cooking process, and the pasta becomes soft and delicious.

But the principles of the two are different. In the former method, it is a chemical process. Baking soda can seriously damage the B vitamins in the flour.

The yeast surface is completed by the biological process of yeast fermentation and improves the nutritional value.

Yeast is divided into fresh yeast and dry yeast. It is an edible, nutrient-rich single-cell microorganism, which is called "inexhaustible nutrient source" by nutrition. In addition to proteins, carbohydrates, and lipids, yeast is also rich in vitamins, minerals, and enzymes. Experiments have shown that the protein content per 1 kg of dry yeast is equivalent to the protein content of 5 kg of rice, 2 kg of soybeans, or 2.5 kg of pork. Therefore, the nutrients contained in taro and bread are 3–4 times higher than those without noodles, and the protein is increased nearly 2 times.

2.4.2 Protect the liver

There is also a strong antioxidant in yeast which can protect the liver and have a certain detoxification effect. Minerals such as selenium and chromium in yeast can resist aging, antitumor, prevent arteriosclerosis, and improve the body's immunity. After fermentation, a phytic acid that affects the absorption of calcium, magnesium, iron, and other elements in the flour can be decomposed, thereby enhancing the absorption and utilization of these nutrients by the human body.

2.4.3 Loose product

Yeast produces a large amount of carbon dioxide in the dough fermentation and is left in the network due to the formation of the gluten network structure, so that the baked food tissue is loose and porous, and the volume is increased.

Yeast also has the effect of increasing gluten expansion, so that the carbon dioxide produced during fermentation can be retained in the dough to improve the gas holding capacity of the dough. This is not the case if a chemical loosening agent is used.

2.4.4 Improve flavor

During the fermentation process, the dough undergoes a series of complex biochemical reactions that produce a unique fermentation aroma of the bread product. At the same time, it forms a unique aroma, rich, and appetizing baking scent. Regarding the effect of umami on the flavor of food, adding savory to the food can improve the overall taste of the food and can also be used to enhance some flavor characteristics of the food, such as persistence, mildness, and thick feeling. The amount of savory added cannot be as high as possible. Studies have shown that MSG has the best flavor-increasing effect at 0.2%–0.8% of the weight of the food. When the relative 5′-IMP (disodium inosine monophosphate) is about 0.02%–0.04%, the equivalent is obtained.

2.4.5 Flavor intensity

But it is also time to consider the ratio of umami to NaCl. If MSG and salt are added to chicken soup or spiced chicken soup, the optimum ratios are 0.33% MSG, 0.83% NaCl and 0.38% MSG, 0.87% NaCl, respectively. Only a certain concentration range gives a pleasant feeling, and too much is counterproductive.

2.4.6 Cover up the odor and light salt effect

In the range of 0.6%–4.0% NaCl content, when the added YE (yeast extract) content is between 0.4% and 3.0%, the salty taste of the solution can be enhanced.

When the NaCl concentration is >7%, the addition of 0.4% or more of YE can weaken the salty taste of the product to varying degrees, and the degree of weakening increases with the increase of NaCl concentration and YE addition.

The characteristics of yeast YE are, pure natural, rich in a variety of amino acids, peptides, and taste nucleotides; delicious, strong aroma, fleshy, and mellow; high temperature, high temperature conditions can give food a better flavor.

2.4.7 Increase nutrition

Because the main component of yeast is protein, it accounts for almost half of the dry matter of yeast, and the human body has sufficient amino acids, especially the lack of lysine in grains. On the other hand, it contains a lot of vitamin B1, vitamin B2, and niacin. Therefore, yeast can improve the nutritional value of fermented foods.

2.5 Mold

Mold is not a taxonomic name. It is a general term for filamentous fungi. Any fungus that has mycelium on a nutrient substrate is collectively referred to as a mold. It includes many fungi that are very differently classified, such as the genera Algae, Ascomycetes, Basidiomycetes, and Deuteromycetes.

The molds commonly used in the food industry include four genera of *Mucor*, *Rhizopus*, *Aspergillus*, and *Geotrichum*.

2.5.1 Mucor

It has a hairy shape, no pseudo roots and lychees, no hyphae, and sporangia are directly produced by mycelium. The mode of reproduction can be directly germinated by ascospores or by spores. *Mucor* produces proteases and thus can break down soybeans. In China, when making bean curd and soybean meal, they use *Mucor* to break down proteins to produce umami. Some species of *Mucor* also have strong saccharifying power and can saccharify starch. *Mucor* in Chinese alcoholic medicine belongs to this category. *Mucor* can also be used in the saccharification and fermentation processes of alcohol and organic acid industrial raw materials.

2.5.2 Rhizopus

The mycelium produces litchi, and the ends of the litchi have pseudo roots. This is the main morphological feature that differs from *Mucor*. *Rhizopus* has a strong saccharification enzyme activity, which can decompose starch into sugar, which is a commonly used saccharification bacteria in the wine industry.

2.5.3 Aspergillus

The mycelium branches and has a transverse septum, and the conidia grow up from the differentiated hyphae (with thick-walled podocytes). The shape, size, color, and ornamentation of conidia are important basis for the identification of *Aspergillus* species.

Aspergillus can decompose organic matter. Widely used in industries such as brewing, biologicalfeed and fertilizer it has a variety of highly active enzyme systems. For example, saccharification bacteria used in winemaking have amylases for liquefying and saccharifying starch, as well as sucrose invertase, maltase, and lactase. Some bacteria can produce strong acid proteases, which can be used to decompose proteins or be used as food digestive agents. The pectinase produced by *Aspergillus niger* is commonly used for juice clarification, naringin, and hesperidinase are used for bitterness in citrus cans or to prevent white precipitates, and glucose oxidase is used for desugar and deoxygenation of foods.

Aspergillus can produce a variety of organic acids such as fumaric acid, lactic acid, and succinic acid. Among them, oxaloacetate and acetyl-CoA are condensed into citric acid. The *Aspergillus* strains which are widely used in the food industry are *Aspergillus oryzae*, *Aspergillus flavus*, *A. oryzae*, and others. These *Aspergillus* play an important role in traditional Chinese foods such as bean paste, soy sauce, white wine, and rice wine.

2.5.4 Geotrichum

Its colonies are like yeast, so they are yeast-like molds. However, it has fungal filaments, and the hyphae have a cross-section. After ripening, the hyphae break into spores. Most of the spores are long cylindrical and have a square or elliptical shape. Generally, whiter *Geotrichum* is commonly found in kimchi, decaying fruits and vegetables, and animal waste. The fungus protein of *Geotrichum candidum* is rich in nutrients and can be used for food or feed.

White mold cheese is a soft cheese wrapped in white mold. The skin is covered with white fungus fluff. The texture is very soft, and the milk is rich. As the mold continues to multiply, the cheese matures from the outside to the inside, and the taste becomes more intense. When it is fully ripe, the mellow flavor inside is elegant and easy to eat. White mold makes the cheese taste smooth, soft, and breaks down the protein into amino acids, adding a sweet taste to the cheese.

Blue cheese, with a history of more than 2000 years, has a spicy flavor. The reproduction of the green mold makes the cheese form a beautiful blue pattern, and the cheese can be eaten at this time. Its taste is more intense and exciting than the white mold cheese. Unlike other cheese varieties, blue cheese is ripening from the center.

2.6 Fermented vegetable food microorganism

Fermented vegetable foods have a long history, and different regions have their own related foods (Swain, Anandharaj, & Ray, 2014). Kimchi can be considered a plant probiotic food with the same health benefits as yogurt as a probiotic food (Park, Jeong, Lee, & Daily, 2014). There are kimchi shadows all over the world, and the flavor varies according to local practices. Among them, Fuling mustard, French pickled cucumber, German sweet and sour cabbage, and the world's three major kimchi. The prepared kimchi is rich in *lactic acid bacteria*, which can help digestion. However, there are certain rules for making kimchi, such as the inability to encounter raw water or oil, otherwise it is prone to corruption and so on. If you eat contaminated kimchi, it is easy to diarrhea or food poisoning.

Fermented vegetable foods have many benefits for human health, especially in the areas of anticancer, antiobesity, and anticaries (Vijayendra & Halami, 2015).

2.6.1 Serofluid dish microorganism

Serofluid dish (or *Jiang shui*, in Chinese) is a traditional food in the Chinese culture for thousands of years. Serofluid dish forms complex and distinct fungal communities structure (Chen, Zhao, & Wu, 2016). Aerobic bacteria such as *Bacillus subtilis*, *S. cerevisiae*, and *Acetobacter* were dominant in the early stage of the natural fermentation, and several acid-resistant yeasts increased. If this stage to continue fermentation will affect product quality. Moreover, if not sterilization preservation, acid-resistant mold will breed and causing rotten products. In the ferrofluid dish sample studies, there are lots of bacterial colonies, such as *Lactobacillus*, *Pediococcus*, and so on. We need to further understand the prokaryotic and eukaryotic organisms and lay a foundation for its industrialization. *Lactic acid bacteria* are the most abundant bacteria, which can produce lactic acid, which is a kind of bacteria that plays a major role in the manufacturing process of fermented dairy products. According to its characteristics of sugar fermentation, it can be divided into homofermentative bacteria and heterofermental fermentation bacteria.

2.6.2 Chinese and Korea kimchi microorganism

Chinese kimchi mainly relies on the fermentation of *lactic acid bacteria* to produce a large amount of lactic acid instead of relying on the osmotic pressure of salt to inhibit spoilage microorganisms. Pickle with low concentration of salt water or use a small amount of salt to pickle all kinds of fresh vegetables, and then fermented by lactic acid bacteria to make a sour pickled product. When the lactic acid content reaches a certain concentration and the product is isolated from the air, it could an achieve the purpose of long-term storage. The salt content in kimchi is 2%–4%, which is a low-salt food. Among them, the most well-known is the Fuling mustard, which uses the unique green cabbage head of Fuling, and is made with a unique processing technique. It is also one of the three famous dishes (sauerkraut, sauerkraut, and bamboo shoots) that are exported to China with French pickles and German sweet and sour cabbage. Its traditional craftsmanship was included in the second batch of national intangible cultural heritage. Inoculation into the mustard, artificially creating the optimum conditions for pickling and fermentation, promoting its rapid growth and reproduction. Thereby enhancing the fermentation of lactic acid to achieve the purpose of inhibiting the invasion of harmful microorganisms and achieving rapid fermentation and curing. The mustard inoculated with beneficial microorganisms was studied to replace most of the salt with a method other than salt, to achieve low-salt fast-pickled fermentation.

Draw the difference between Korean and Chinese kimchi for future use in the confirmation of kimchi origin (Lee, Cho, Han, & Lee, 2010).

Kimchi is probably Korea's most famous traditional fermented food (Park et al., 2016), and about 200 types of kimchi are available in Korea (Jang, Chung, Yang, Kim, & Kwon, 2015). Kimchi types vary according to vegetables, spices, and other ingredients used and methods of preparation (Lee, 1991). Kimchi possesses antiinflammatory, antibacterial, probiotic properties, cholesterol reduction, and antiaging properties (Patra, Das, Paramithiotis, & Shin, 2016). Kimchi is a representative of Korean traditional food, mainly fermented by *lactic acid bacteria*, and is a potential source of *lactic acid bacteria* and probiotics. Kimchi *lactic acid bacteria* have become a research hotspot of Korean scholars, and its research methods mainly include cultivating both dependent and nonculture dependent types. The genera *Lactobacillus and Weissella* are identified as the dominant lactic acid genera in Korean kimchi, and the fermentation type mainly belongs to obligate heterotypic fermentation and facultative heterotypic fermentation. The distribution and succession of *lactic acid bacteria* in kimchi are mainly affected by factors such as raw materials for kimchi, kimchi varieties, fermentation temperature, and salt concentration (Table 24.2).

TABLE 24.2 Fermented vegetable-based foods microorganism.

Food	Country	Ingredients	Microorganisms	Health/Nutrition benefits	References
Dhamuoi	Vietnam	Cabbage, various vegetables	*Leuconostoc mesenteroides*, *Lactobacillus plantarum*	Increased nutritional quality	Lee (1997)
Kawal	North Africa	Vegetables	*L. plantarum*	Increased nutritional quality	Oyewole (1997)
Kimchi	Korea	Korean cabbage, radish, various vegetables	*L. mesenteroides*, *Lactobacillus brevis*, *L. plantarum*	Antimicrobial properties, antitumor activity, prevent constipation	Lee (1997), Kwon & Young-Kyung, (2003)
Sauerkraut	Germany	Cabbage	*L. mesenteroide*, *L. brevis*, *L. plantarum*, *Pediococcus cerevisiae*	Source of vitamins and fiber	Steinkraus (1983), Holzapfel, Haberer, and Snel (1998)
Tsukemono	Japan	Vegetables, rice flour	*Lactic acid bacteria*	Increased nutritional quality	Claude (1985)

2.6.3 FSP, fermented soy products microorganism

Soybean has many compounds with a variety of biological properties that potentially benefit human health (Choi et al., 2011), which could serve as a great substitute for meat proteins (Xu, Du, & Xu, 2015). In Japan, fermented soybean products are known as natto and miso; in Korea, cheonggukjang; and in China, douche (Mani & Ming, 2017). Natto originated in China, similar to Chinese fermented beans and strange beans, and is now a common Japanese food. The ancient book records that China has been producing natto since the Qin and Han dynasties, and it was originally developed in China. Among them, the salty natto was developed in Japan in the Nara and the Heian era. The extracellular product of natto fermentation is a protein kinase that can dissolve serine in cross-linked fibrin, nattokinase, which can effectively dissolve thrombus and has various physiological functions, such as lowering blood viscosity, inhibiting platelet coagulation, and lowering blood fat. Nattokinase has obvious advantages as a health food additive or directly used in the pharmaceutical industry, lowering cholesterol, improving blood circulation, and maintaining the normal morphology and function of blood cells.

2.6.4 Korean soybean paste stew microorganism

Doenjang is a fermented soybean paste used primarily as soup base and as a dipping agent for vegetables and meat in Korea (Kim & Lee, 2003). Doenjang is recognized as a nutritious food (Nam, Lee, & Lim, 2012). Doenjang has been reported to exhibit antioxidant, fibrinolytic, anticancer, and antiobesity effects (Jang, Kim, Han, & Kim, 2014).

2.6.5 Natto microorganism

Natto is a traditional Japanese food made by boiling or steaming soybeans and fermenting them with the *B. subtilis natto* (Milner & Makise, 2002). Natto is a traditional food (fermented soybean) in Japan. It produces many kinds of active components. It can prevent blood clotting, anticancer, and lower the blood pressure (QI Hai ping, 2003). *Bacillus natto* is an aerobic Gram-positive probiotic with strong heat, acid–base stability, and rapidly develops into vegetative cells after entering the intestine, inhibiting *Enterobacter*, *Enterococcus*, *pathogenic bacteria*, etc. Reproductive, they promote the growth of anaerobic bacteria such as *Bifidobacteria*, *Bactobacilli,* and *Clostridium*, regulate microecological balance, metabolize and secrete a variety of enzymes that promote the digestion and absorption of nutrients, strengthen digestive function, and stimulate the immune system in the body. It produces antibiotics such as interferon and nattokinase with high potency of thrombolysis, which enhances cellular immune function in animals.

B. natto is a subspecies of *B. subtilis*. It is a kind of aerobic and endogenous antireverse rod-shaped bacteria. It has no pathogenicity and has only a single layer of extracellular membrane, which can produce a variety of antibiotics. Enzyme has a broad-spectrum antibacterial activity and strong resistance to stress. Under extreme conditions, it is also possible to induce endogenous cells with strong stress resistance.

Spore natto is a kind of heat resistance and acid resistance of beneficial bacteria. Survive gastric acid for 4 hours was 100% survival rate, with a strong ability to inhibit pathogens. It is most suitable for a variety of beneficial bacteria. Once in the small intestine, it can change the ecology of the human gut flora strains after oral administration; it helps normalize the function of the digestive tract, making bowel movements smooth, and changes the body physiological environment.

This strain has a function of generating an acid, intestinal flora, and enhancing immune response of an animal cell. It can also produce a variety of proteases (especially the alkaline proteases), glucoamylase, lipase, amylase, and degradation of plant and certain complex carbohydrates in the feed to improve the feed conversion rate.

2.6.6 Tempeh microorganism

Tempeh is a fermented soy food bound together by a dense mycelium of fragrant white *Rhizopus* mold into compact cakes (Wang, 1984). Tempeh is gaining popularity in Asia and the rest of the world because it is an economical, nutritious, healthy, and easy-to-prepare food (Mani & Ming, 2017). Tempeh as a fermented soybean product also contains folate, which is beneficial for cognitive (Hogervorst, Mursjid, & Priandini, 2011).

2.6.7 Stinky tofu microorganism

Stinky tofu is a well-known and popular traditional fermented Chinese snack (Chao, Tomii, & Sasamoto, 2008). Tofu is a fundamental part of Asian food culture. Currently, its consumption and popularity are growing rapidly in the West (Serrazanetti, Ndagijimana, & Miserocchi, 2013). Stinky tofu is rich in plant-based *lactic acid bacteria*, which has a good regulation of intestinal and stomachic effects.

Compared with animal *lactic acid bacteria*, plant *lactic acid bacteria* have stronger acid and bile-tolerant abilities and can survive in the harsh environment of uneven nutrition. Therefore, the survival rate of human intestinal tract is higher and the colonization ability is stronger. Therefore, plant *lactic acid bacteria* products have an advantage in improving the intestinal environment and improving human immunity (Table 24.3).

TABLE 24.3 Fermented legume-based foods microorganism.

Food	Country	Ingredients	Microorganisms	Health/Nutrition benefits	References
Bhallae	India	Black gram	*Lactic acid bacteria, yeasts*	Increased nutritional quality	Prajapati & Nair, (2003)
Dawadawa	Nigeria	Locust bean (*Parkia biglobosa*) seeds	*Bacillus* sp.	Increased nutritional quality	Steinkraus (1997), Menash (1997)
Doenjang	Korea	Soybeans	*Aspergillus oryzae*	Increased nutritional quality	Kwon & Young-Kyung, (2003)
Falafel	Middle East	Chick peas		Increased nutritional quality	Blandino et al. (2003), Steinkraus (1983)
Hama-natto	Japan	Soybeans	*A. oryzae*, *Streptococcus* sp., *Pediococcus* sp.	Degradation of fat, protein, so that yogurt is more conducive to digestion and absorption	Lee (1997)
Iru	West Africa	Locust bean (*P. biglobosa*) seed	*Bacillus* sp.	Increased nutritional quality	Lee (1997)
Kecap	Indonesia	Soybeans	*A. oryzae*	Increased nutritional quality	Lee (1997)
Kenima	India, Nepal	Soybeans		Increased nutritional quality	Prajapati & Nair, (2003)
Kinema	India, Nepal	Soybeans	*Bacillus subtilus*, *Enterococcus faecium*, *Candida parapsilosis*, *Geotrichum candidum*	Increased nutritional quality	Lee (1997), Sarkar, Hasenack, and Nout (2002)
Meitauza	China, Taiwan	Soybeans	*Actinomucor elegans*	Increased nutritional quality	Prajapati & Nair, (2003)
Meju	Korea	Soybeans	*A. oryzae*, *Rhizopus* sp.	Increased nutritional quality	Prajapati & Nair, (2003)
Miso	Japan	Soybeans	*A. oryzae*, *Torulopsis etchellsii*, *Lactobacillus* sp.	Increased nutritional quality	Blandino et al. (2003)
Natto	Japan	Soybeans	*Bacillus (subtilis) natto*	Possible stimulation of immune system	Steinkraus (1983), Kiuchi & Hosoi, (2003)
Ogiri-igbo	Nigeria	Castor beans (*Ricinus communis*)		Increased nutritional quality	Lee (1997)
Ogiri-nwan	Nigeria	Fluted pumkin beans (*Telfaria occidentale*)		Increased nutritional quality	Lee (1997)
Ontjom	Indonesia	Peanut cake	*Neurospora intermedia*, *Rhizopus oligosporus*	Decomposes the protein in the tofu into soluble small-molecule polypeptides and amino acids, and breaks down the fat into glycerol and fatty acids, so that the fermented bean produces aromatic substances or umami-degraded protein decomposition products	Prajapati & Nair, (2003)
Papadam	India	Black gram	*Saccharomyces* sp.	Making foods and wines such as bread and taro, used as eukaryotic model organisms in modern molecular and cellular biology	Blandino et al. (2003)
Puda or pudla	India	Bengal gram, mung, beans, wheat	*Lactic acid bacteria, yeasts*	Increased nutritional quality	Prajapati & Nair, (2003)

Soumbara	Ivory Coast	Locust bean (*P. biglobosa*) seeds	*Bacillus* sp.	It has the advantage of occupying space and inhibiting the growth and reproduction of harmful microorganisms such as harmful bacteria and pathogens	Steinkraus (1997)
Sufu or furu (wide variety of other names in China and elsewhere)	China, Japan, Vietnam, Thailand, Philippines	Soybeans	*Actinomucor elegans*, *Aeromonas taiwanensis*, *Mucor sufu* *Mucor wutungkiao*, *Rhizopus* sp.	Increased nutritional quality	Steinkraus (1983), Han, Rombouts, and Nout (2001)
Tamiah	Egypt	Beans		Increased nutritional quality	Claude (1985)
Tempeh (various name variations including tempe gembus, tempe benguk, tempe kedele)	Indonesia	soybeans	*Rhizopus mold*	Decomposes the protein into soluble small-molecule polypeptides and amino acids, and breaks down the fat into glycerol and fatty acids, so that the fermented bean produces aromatic substances or umami-degraded protein decomposition products	Abdel Gadir, Mohamed, Abd-el-Malek, Ahmad, & Arroyo, (1983)
Tempe kecipir	Indonesia	Winged beans (*Psophocarpus tetragonolobus*)	*Rhizopus mold*	Decomposes the protein into soluble small-molecule polypeptides and amino acids, and breaks down the fat into glycerol and fatty acids, so that the fermented bean produces aromatic substances or umami-degraded protein decomposition products	Abdel Gadir, Mohamed, Abd-el-Malek, Ahmad, & Arroyo, (1983)
Thua-nao	Thailand	soybeans	*B. subtilis*	Stimulate the growth and development of animal (human) immune organs, activate T, B lymphocytes, improve immunoglobulin and antibody levels, enhance cellular and humoral immune functions, and improve group immunity	Lee (1997), Sarkar et al. (2002)
Vada or vadai	India	Black gram (*Phaseolus mungo*), rice	*Pediococcus* sp., *Leuconostoc* sp., *Streptococcus* sp.	Degradation of fat, protein, so that yogurt is more conducive to digestion and absorption	Blandino et al. (2003), Paradkar, Singhal, and Kulkarni (2002)
Waries	India	Black gram flour	*Candida* sp., *Saccharomyces* sp.	Making foods and wines such as bread and taro, used as eukaryotic model organisms in modern molecular and cellular biology	Prajapati & Nair, (2003)

2.7 Microorganism in fermented dairy products

2.7.1 Cheese

Cheese, a fermented milk product, has similar properties to common yogurt. It is made by fermentation. It also contains *lactic acid bacteria* that can be used for health care, but the concentration of cheese is higher than that of yogurt. The nutritional value is therefore more abundant. Each kilogram of cheese is concentrated from 10 kg of milk. It is rich in protein, calcium, fat, phosphorus, and vitamins. It is a natural food. In terms of craftsmanship, cheese is fermented milk; in terms of nutrition, cheese is concentrated milk. Flavor development in dairy fermentations, most notably cheeses, results from a series of (bio)chemical processes in which the starter cultures provide the enzymes (Smit, Smit, & Engels, 2005).

White mold cheese is a soft cheese wrapped in white mold. The skin is covered with white fungus fluff. The texture is very soft, and the milk is rich. As the mold continues to multiply, the cheese matures from the outside to the inside, and the taste becomes more intense. When it is fully ripe, the mellow flavor inside is elegant and easy to eat. White mold makes the cheese taste smooth, soft, and breaks down the protein into amino acids, adding a sweet taste to the cheese.

Blue cheese, with a history of more than 2000 years, has a spicy flavor. The reproduction of the green mold makes the cheese form a beautiful blue pattern, and the cheese can be eaten at this time. Its taste is more intense and exciting than the white mold cheese. Unlike other cheese varieties, blue cheese ripens from the center.

2.7.2 Yogurt

Yogurt is a kind of milk product that is made from milk as raw material, pasteurized and then added with beneficial bacteria (fermenting agent) to the milk, and then cooled and then filled. At present, most of the yoghurt products on the market are solidified, stirred, and added with various fruit juice jams and other fruit flavors. Yogurt not only retains all the advantages of milk but also some aspects of the process of processing and development to avoid shortcomings, becoming more suitable for human nutrition and health products. The current milk consumption in Indonesia is approximately 15 L per capita per year, and more than 70% of milk and milk products are imported from other countries such as Australia and New Zealand (Sumarmono, Sulistyowati, & Soenarto, 2015). There are therapeutic and preventive effects of yogurt on diseases such as cancer, infection, gastrointestinal disorders, and asthma (Meydani & Ha, 2000). *Lactic acid bacteria* are also widely found in livestock, poultry, many foods, materials, and a few clinical samples. They can not only improve the nutritional value of food, improve the flavor of food, improve the preservation and added value of food but also the special physiological activity and nutritional function of *lactic acid bacteria* are increasingly attracting people's attention. Numerous studies have shown that *lactic acid bacteria* can regulate the normal flora of the gastrointestinal tract, maintain microecological balance, improve food digestibility and bioprice, reduce serum cholesterol, control endotoxin, inhibit the growth and reproduction of spoilage bacteria in the intestine, and produce spoilage products. Nutrients stimulate tissue development and thus play a role in the body's nutritional status, physiological functions, cell infections, drug effects, toxicity, immune response, tumorigenesis, aging process, and sudden emergency response. The physiological function of *lactic acid bacteria* is closely related to the life activities of the body. It can be said that if *lactic acid bacteria* stop growing, it is difficult for humans and animals to survive healthily. Because of this, *lactic acid bacteria* are widely used in many industries such as light industry, food, medicine, and feed industry.

2.8 Fermented meat and fish

Fermented meat products are defined as meats that are deliberately inoculated during processing to ensure sufficient control of microbial activity to alter the product characteristics (Ricke, Koo, & Keeton, 2013). The genera most commonly used for meat starter cultures are *Lactobacillus*, *Pediococcus*, *Micrococcus*, and *Staphylococcus*, *Pediococcus*, and *Lactobacillus*, and other LAB are preferred when acid production is of primary importance (Ricke, Diaz, & Keeton, 2007) (Table 24.4).

2.9 Microorganism in fermented grain–based foods

Over the generations, this pioneering practice of food fermentation has expanded and improved to preserve and fortify the available food resources (Ray, Ghosh, & Singh, 2016). By utilizing microorganisms and their enzymes, cereal grains can be transformed into value-added products (Badrie, 2010) (Table 24.5).

2.10 Probiotics

Probiotics are usually defined as microbial food supplements with beneficial effects on the consumers. Most probiotics fall into the group of organisms known as lactic-acid-producing bacteria and are normally consumed in the form of yogurt,

TABLE 24.4 Fermented fish- and meat-based foods.

Food	Country	Ingredients	Microorganisms	Health/Nutrition benefits	References
Anchovy	Mediterranean countries, Argentina	Anchovy			Claude (1985)
Burong dalag	Philippines	Fish (dalag), rice	*Leuconostoc mesenteroides*, *Pediococcus cerevisiae*, *Lactobacillus plantarum*, *Streptococcus faecalis*, *Micrococcus* sp.	Antimicrobial properties, antitumor activity, prevent constipation	Steinkraus (1983), Orillo and Pederson (1968)
Burong hipon	Philippines	shrimp			Orillo and Pederson (1968)
Burong isda	Philippines	Fish, rice	*Lactobacillus brevis*, *Streptococcus* sp.	Degradation of fat, protein, so that yogurt is more conducive to digestion and absorption	Oyewole (1997)
Kungchao	Thailand	Shrimp, rice	*P. cerevisiae*		Oyewole (1997)
Nahm	Thailand	Pork, rice	*Pediococcus* sp., *P. cerevisiae*, *L. plantarum*, *L. brevis*, *Lactobacillus lactis*	Antimicrobial properties, antitumor activity, prevent constipation	Steinkraus (1983), Noonpakdee, Santivarangkna, and Jumriangrit (2003)
Narezushi	Japan	Fish, millet	*L. mesenteroides*, *L. plantarum*	Antimicrobial properties, antitumor activity, prevent constipation	Lee (1997)
Nem-chua	Vietnam	Pork, rice	*Pediococcus* sp., *Lactobacillus* sp.	It regulates blood lipids, protects and repairs liver from hyperlipidemia, and enhances the body's antioxidant capacity	Lee (1997)
Pla-ra	Thailand	Fish, rice	*Pediococcus* sp.		Lee (1997)
Sai-krok-prieo	Thailand	Pork, rice	*L. plantarum*, *Streptococcus salivarius*, *Pediococcus pentosaceus*	Antimicrobial properties, antitumor activity, prevent constipation	Lee (1997)
Salami	Europe	Pork	*Lactobacillus* sp., *Mircococcus*	It regulates blood lipids, protects and repairs liver from hyperlipidemia, and enhances the body's antioxidant capacity	Lee (1997)
Sikhae	Korea	Fish, millet	*L. mesenteroides*, *L. plantarum*	Antimicrobial properties, antitumor activity, prevent constipation	Lee (1997)
Surstromming	Scandinavian countries	Herring			Lee (1997)
Nem chua	Vietnam	Pork dish			Claude (1985)

fermented milks, or other fermented foods (Parvez, Malik, Ah Kang, & Kim, 2006). The development of probiotics, which are living bacteria or yeasts used to confer a health benefit on the host (McFarland, 2015) (Tables 24.6 and 24.7).

2.11 Bacteria

The morphology of bacteria often changes with changes in the living environment. However, under certain environmental conditions, various bacteria have a certain shape, and the basic forms are spherical, rod-shaped, and spiral. Most cocci

TABLE 24.5 Fermented cereals microorganism.

Food	Country	Ingredients	Microorganisms	Health/Nutrition benefits	References
Lao-zao	China, Indonesia	Rice	*Rhizopus oryzae*, *Rosa chinensis*, *Chlamydomucor*, *A. oryzae*, *Saccharomycopsis*	It secretes a glucoamylase that converts starch to glucose	Blandino et al. (2003)
Chinese *yeast*	China	Maize	*Mucoraceous molds*, *yeasts*	As a food nutrition enhancer in baby foods and health foods	Blandino et al. (2003)
Mantou	China	Wheat flour	*Saccharomyces* sp.	Making foods and wines such as bread and taro, used as eukaryotic model organisms in modern molecular and cellular biology	Blandino et al. (2003)
Minchin	China	Wheat gluten	*Paecilomyces* sp., *Aspergillus* sp., *Cladosporium* sp., *Fuarium* sp.		Prajapati & Nair, (2003)
Red *yeast* rice or koji or hongqu	China, Philippines	Rice	*Monascuc purpureus Went*	Improved digestion, lowers blood lipid levels	Claude (1985)
Shamsy bread	Egypt	Wheat flour	*Yeasts*		Prajapati & Nair, (2003)
Nan	India, Pakistan, Afghanistan, Iran	Wheat flour	*Lactic acid bacteria*, *Saccharomyces cerevisiae*		Blandino et al. (2003)
Mawe	South Africa	Maize	*Lactobacillus brevis*, *Lactobacillus fermentum*, *Leuconostoc mesenteroides*, *Lactobacillus lactis* subsp. *lactis*, *Pediococcus pentosaceus*, *Weissella confusa*, *yeasts*		Oyewole (1997), Blandino et al. (2003), Muyanja, Narvhus, and Treimo (2003)

TABLE 24.6 Bacteria commonly found in probiotic food products.

Genus	Species	Foods	References
Lactobacillus	*Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Shirota johnsonii crispatus*, *Lactobacillus rhamnosus GG gasseri reuteri plantarum*	Yogurt	(Wu, Tsai, Hwang, & Chiu, 2012)
Bifidobacterium	*Bifidobacterium bifidum*, *Bifidobacterium animalis*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Bifidobacterium adolescentis*	Dairy products	Centanni et al. (2014)
Enterococcus	*Enterococcus faecium*, *Enterococcus faecalis*	Food adjuvant	Weaver (2015)
Lactococcoccus lactis	*L. lactis*	Dairy products and plant products	Chen, Shen, Ingvar Hellgren, Ruhdal Jensen, and Solem (2015)
Leuconostoc	*Leuconostoc mesenteroides*	Fruit and vegetable	
Streptococcus	*Streptococcus thermophilus cremoris*	Dairy products	Menezes et al. (2016)
Pediococcus	*Pediococcus acidilactici*	Vegetables and food	Attri et al. (2015)
Other	*Escherichia coli*, *Propionibacterium freudenreichii*, *Saccharomyces cerevisiae*		

TABLE 24.7 The function and characteristics of the main probiotics in fermented food.

Strains	Source	Characteristic and functional properties	References
Lactic acid bacteria	Livestock, poultry, food, materials	Promote animal growth, regulate normal gastrointestinal flora, maintain microecological balance, thereby improve gastrointestinal function; improve food digestibility and biopotency; lower serum cholesterol, control endotoxin; inhibit growth of spoilage bacteria in the intestine: improve the body Immunity, etc.	Thakur, Tomar, and De (2016)
Bacillus subtilis	Soil and corrupt organic matter	Help digestion; can be mixed with a variety of strains, play an important role in agricultural production; animal husbandry, poultry, special animals and pet farming, aquaculture; municipal and industrial wastewater treatment	Torres, Brandan, Petroselli, Erra-Balsells, and Audisio (2016)
Yeast	In the air, in the soil, in the water, in the animal	Making noodles; protecting the liver; loosening the product; improving the flavor	Rossouw and Bauer (2016)
Bifidobacterium	Feces of breast milk nutrition	Free radicals, increase host antioxidant enzyme activity, reduce serum and liver malondialdehyde content, thereby alleviating oxidative damage in the body, delaying aging; improving diseases caused by intestinal flora imbalance, such as constipation, diarrhea, etc.;—Galactosidase, which relieves lactose intolerance; inhibits the growth of spoilage bacteria and decomposes carcinogens to fight cancer	O'Callaghan, Bottacini, O'Connell Motherway, and van Sinderen (2015)
Actinomycesbovis	Soil, air, and water	Many of the complex secondary metabolites produced have pharmaceutical and plant protection uses	

are 0.5–2.0 m in diameter, and bacilli are generally 0.5–1 μm in length and 1–5 μm in length. Spore-forming bacteria are generally slightly larger than spore-free bacteria. The mass of the bacteria is about 1×10 to 1×10 mg. Bacteria have a large surface-area-to-volume ratio, so their metabolism is very active. For example, the surface area/volume ratio of *Lactobacillus* is 120,000, and the surface area/volume ratio of a person weighing 100 kg is only 0.3; the lactic acid produced by *Lactobacillus* for 1 hour is about 1000–10,000 times its body weight, and one person wants to obtain 1000 times. It takes 40 years for the sugar metabolite of its body weight. The use of bacteria, an active metabolic function, creates a variety of useful products in fermented foods.

Bacterial reproduction is carried out by cell division, which is very fast, with the shortest generation time of only 10 minutes and the slow growth of bacteria only a few hours.

3 Function and application of food microorganisms

3.1 Health function of probiotics microorganisms

The microorganisms in most fermented foods are probiotics. When the human body is full of enough probiotics, people will be in a healthy state, but once the flora in the body is out of balance, such as when the proportion between the bacteria changes greatly or exceeds the normal value, then a series of illnesses such as diarrhea, allergies, poor appetite, fatigue, and low immunity will follow, and the health of the human body will turn red. At this time, the mixed bacteria fermentation drink is added to help the body flora balance in order to make people regain their health.

1. Prevention or improvement of diarrhea: Poor eating habits or taking antibiotics will break the balance of intestinal flora, leading to diarrhea. Supplementation of probiotics helps to balance the intestinal flora and restore normal intestinal pH and relieve diarrhea.
2. Relieve lactose symptoms: *Lactobacillus* can help the body break down lactose, relieve diarrhea, flatulence, and other symptoms, and can be eaten with milk.
3. Prevention of reproductive system infection: *L. acidophilus* in yogurt and Ricardo antibacterial spray can inhibit the reproduction of *Candida albicans* in the vagina. Double-blind controlled trials in Europe confirmed this. The patient supplemented the body with 10 mL of active probiotics every day. The results showed that the incidence of reproductive

system infections was greatly reduced. Long-term supplementation with active probiotics in the body can help repair the microcirculation of the reproductive system.

4. Enhance human immunity: There is a very developed immune system in the intestine. Probiotics can regulate hypo- or excessively high immune activity to normal by stimulating immune function in the intestine. The immunomodulatory role of probiotics is also believed to contribute to anticancer and allergic diseases.
5. Promote intestinal digestive system health: Probiotics can inhibit the proliferation of harmful bacteria in the intestine, reduce toxins, promote intestinal peristalsis, thereby improving intestinal function and improving defecation.
6. Lower serum cholesterol: Europe's Caucasus Mountains, the Mediterranean coast is a famous longevity town, locals often drink homemade yogurt, rarely suffering from diabetes, cardiovascular disease, and obesity, and a large number of scientific studies confirmed that this is rich with probiotics in yogurt. These probiotics lower serum cholesterol levels. In addition, long-term supplementation with probiotics helps prevent bone loss and prevent osteoporosis.
7. Help absorb nutrients: If you take probiotics every day, you can not only produce harmful bacteria in the intestines but also provide a good environment for the beneficial bacteria in the intestines to create a healthy intestinal tract.

Patients often take health foods containing probiotics to prevent and treat diarrhea. Normal human intestines inhabit more than 500 kinds of tens of billions of different bacteria, which in most cases are mutually restricted, coexisting, and coprosperous. Once the balance of the intestinal flora is broken, it can cause diarrhea. Second, the abuse of antibiotics can also cause diarrhea. Some medical centers in Europe have tried to treat traveller's diarrhea with oral liquids containing *Lactobacillus*, *Bifidobacterium*, and inulin as the main ingredients and have achieved good results.

3.2 Diseases caused by intestinal flora imbalance

A healthy person has a wide variety of microorganisms in the gastrointestinal tract. These microorganisms are called intestinal flora. The intestinal flora is combined in a certain proportion, and the bacteria are mutually restricted and interdependent, forming an ecological balance in quality and quantity. Once the environment changes inside and outside the body, especially long-term application of broad-spectrum antibiotics, sensitive intestinal bacteria are inhibited. The uninhibited bacteria multiply and multiply, causing the flora to be dysfunctional, and its normal physiological combination is destroyed, and the pathological combination and the clinical symptoms are called intestinal flora disorders.

4 Prospect

Microorganisms are the soul of food fermentation. The development of microbial research is related to the development of fermented foods in the future. Therefore, research on the microbial diversity of fermented foods should be emphasized. The normal production of fermented food is a process of providing a good living environment for microorganisms and allowing them to grow up. The normal and orderly work of these jobs is the prerequisite for ensuring the success of fermented food production. Strengthening the research on microbial diversity is to make the quality of fermented foods is guaranteed, so the microbial diversity of fermented foods is very important. Strengthening the research on the microbial diversity of fermented foods is an essential part of the development of fermented foods at this stage. Only the necessary improvement of food fermentation technology and avoiding some risks in the food production process can make food fermentation have a good development momentum. The study of microbial diversity has an important impact on the normal fermentation of food. In the food fermentation, it is necessary to properly culture the microorganisms, so as to improve the working efficiency of the microorganisms, increase the yield of the fermented food, and enable the fermentation of the food. Faster and safer, food fermentation technology can also make a breakthrough.

References

Abdel Gadir, A., Mohamed, M., Abd-el-Malek, Y., Ahmad, I., & Arroyo, P. (1983). Indigenous fermented foods involving an acid fermentation: Preserving and enhancing organoleptic and nutritional qualities of fresh foods. *Microbiology Series (USA)*, *6*, 21–28.

Achi, O. (2005). The potential for upgrading traditional fermented foods through biotechnology. *African Journal of Biotechnology*, *4*(5), 375–380.

Attri, P., Khaket, T. P., Jodha, D., Singh, J., & Dhanda, S. (2015). Biochemical, kinetic, and in silico characterization of DING protein purified from probiotic lactic acid bacteria *Pediococcus acidilactici* NCDC 252. *Biotechnology and Applied Biochemistry*, *175*(2), 1092–1110.

Badrie, N. (2010). Cereal-based grain products: Fermented indigenous grains. In *Encyclopedia of biotechnology in agriculture and food* (pp. 138–142). Taylor & Francis: CRC Press.

Baroudi, A. A., & Collins, E. B. (1976). Microorganisms and characteristics of Laban. *Journal of Dairy Science*, *59*(2), 200–202.

Blandino, A., Al-Aseeri, M. E., & Pandiella, S. S. (2003). Cereal-based fermented foods and beverages. *Food Research International*, *36*(6), 527–543.

Claude, A. (1985). *Les aliments fermentés traditionnels. Une richesse méconnue*. Paris: Terre Vivante.
Caplice, E., & Fitzgerald, G. F. (1999). Food fermentations: Role of microorganisms in food production and preservation. *International Journal of Food Microbiology*, *50*(1), 131–149.
Centanni, M., Turroni, S., Rampelli, S., Biagi, E., Quercia, S., Consolandi, C., et al. (2014). *Bifidobacterium animalis* ssp. lactis BI07 modulates the tumor necrosis factor alpha-dependent imbalances of the enterocyte-associated intestinal microbiota fraction. *FEMS Microbiology Letters*, *357*(2), 157–163.
Chao, S. H., Tomii, Y, & Sasamoto, M. (2008). *Lactobacillus capillatus* sp. nov., a motile bacterium isolated from stinky tofu brine. *International Journal of Systematic and Evolutionary Microbiology*, *58*(11), 2555–2559.
Chen, J., Shen, J., Ingvar Hellgren, L., Ruhdal Jensen, P., & Solem, C. (2015). Adaptation of *Lactococcus lactis* to high growth temperature leads to a dramatic increase in acidification rate. *Scientific Reports*, *5*, 14199.
Chen, P., Zhao, Y., & Wu, Z (2016). Metagenomic data of fungal internal transcribed spacer from serofluid dish, a traditional Chinese fermented food. *Genomics Data*, *7*, 134–136.
Choi, J., Kwon, S. -H., Park, K. -Y., Yu, B. P., Kim, N. D., Jung, J. H., et al. (2011). The anti-inflammatory action of fermented soybean products in kidney of high-fat-fed rats. *Journal of Medicinal Food*, *14*(3), 232–239.
Farnworth, E. R. (2003). *Handbook of fermented functional foods* (pp. 287–304). Boca Raton: CRC Press.
Gadaga, T. H., Mutukumira, A. N., Narvhus, J. A., & Feresu, S. B. (1999). A review of traditional fermented foods and beverages of Zimbabwe. *International Journal of Food Microbiology*, *53*(1), 1–11.
Han, B. Z., Rombouts, F. M., & Nout, M. J. R. (2001). A Chinese fermented soybean food. *International Journal of Food Microbiology*, *65*(1), 1–10.
Hesseltine, C., & Wang, H. L. (1967). Traditional fermented foods. *Biotechnology and Bioengineering*, *9*(3), 275–288.
Hogervorst, E., Mursjid, F., & Priandini, D. (2011). Borobudur revisited: Soy consumption may be associated with better recall in younger, but not in older, rural Indonesian elderly. *Brain Research*, *1379*(1379), 206.
Holzapfel, W. H., Haberer, P., & Snel, J. (1998). Overview of gut flora and probiotics. *International Journal of Food Microbiology*, *41*(2), 85–101.
Jang, D. -J., Chung, K. R., Yang, H. J., Kim, K. -S., & Kwon, D. Y. (2015). Discussion on the origin of kimchi, representative of Korean unique fermented vegetables. *Journal of Ethnic Foods*, *2*(3), 126–136.
Jang, S. -E., Kim, K. -A., Han, M. J., & Kim, D. -H. (2014). Doenjang, a fermented Korean soybean paste, inhibits lipopolysaccharide production of gut microbiota in mice. *Journal of Medicinal Food*, *17*(1), 67–75.
Kim, S. -H., & Lee, K. -A. (2003). Evaluation of taste compounds in water-soluble extract of a doenjang (soybean paste). *Food Chemistry*, *83*(3), 339–342.
Kiuchi, K., & Hosoi, T. (2003). Natto – A food made by fermenting cooked soybeans with *Bacillus subtilis* (natto). In *Handbook of fermented functional foods* (pp. 227–250). RCRC Press LLC.
Kwon, H., & Young-Kyung, L. K. (2003). Korean fermented foods. In *Handbook of fermented functional foods* (pp. 287–304). Taylor & Francis.
Lee, C. -H. (1997). Lactic acid fermented foods and their benefits in Asia. *Food Control*, *8*(5–6), 259–269.
Lee, M. -J., Cho, K. -H., Han, E. -S., & Lee, J. -H. (2010). Bacterial diversity in the initial fermentation stage of Korean and Chinese kimchi. *Microbiology and Biotechnology Letters*, *38*(2), 207–215.
Lee, Y. C. (1991). Kimchi: The famous fermented vegetable product in Korea. *Food Reviews International*, *7*(4), 399–415.
Mani, V., & Ming, L. (2017). Tempeh and other fermented soybean products rich in isoflavones. In *Fermented foods in health and disease prevention* (pp. 453–474). United Kingdom: Elsevier.
Marteau, P., Flourie, B., & Pochart, P. (1990). Effect of the microbial lactase (EC 3.2. 1. 23) activity in yoghurt on the intestinal absorption of lactose: An in vivo study in lactase-deficient humans. *British Journal of Nutrition*, *64*(01), 71–79.
McFarland, L. V. (2015). From yaks to yogurt: The history, development, and current use of probiotics. *Clinical Infectious Diseases*, *60*(Suppl_2), S85–S90.
Menash, M. (1997). Fermentation-the key to food safety assurance in Africa? *Food Control*, *8*(5–6), 271–278.
Menezes, A. P., Azevedo, J., Leite, M. C., Campos, L. C., Cunha, M., Carvalho Mda, G., et al. (2016). Nasopharyngeal carriage of *Streptococcus pneumoniae* among children in an urban setting in Brazil prior to PCV10 introduction. *Vaccine*, *34*(6), 791–797.
Meydani, S. N, & Ha, W. K. (2000). Immunologic effects of yogurt. *The American Journal of Clinical Nutrition*, *71*(4), 861–872.
Milner, M., & Makise, K. (2002). Natto and its active ingredient nattokinase: A potent and safe thrombolytic agent. *Alternative & Complementary Therapies*, *8*(3), 157–164.
Muyanja, C., Narvhus, J. A., & Treimo, J. (2003). Isolation, characterisation and identification of lactic acid bacteria from Bushera: A Ugandan traditional fermented beverage. *International Journal of Food Microbiology*, *80*(3), 201–210.
Nam, Y. -D., Lee, S. -Y., & Lim, S. -I. (2012). Microbial community analysis of Korean soybean pastes by next-generation sequencing. *International Journal of Food Microbiology*, *155*(1–2), 36–42.
Noonpakdee, W., Santivarangkna, C., & Jumriangrit, P. (2003). Isolation of nisin-producing *Lactococcus lactis* WNC 20 strain from nham, a traditional Thai fermented sausage. *International Journal of Food Microbiology*, *81*(2), 137–145.
O'Callaghan, A., Bottacini, F., O'Connell Motherway, M., & van Sinderen, D. (2015). Pangenome analysis of *Bifidobacterium longum* and site-directed mutagenesis through by-pass of restriction-modification systems. *BMC Genomics*, *16*, 832.
Oelschlaeger, T. A. (2010). Bacteria as tumor therapeutics? *Bioengineered Bugs*, *1*(2), 146–147.
Orillo, C. A, & Pederson, C. S. (1968). Lactic acid bacterial fermentation of burong dalag. *Applied Microbiology*, *16*(11), 1669–1671.
Oyewole, O. B. (1997). Lactic fermented foods in Africa and their benefits. *Food Control*, *8*(5–6), 289–297.
Paradkar, M. M., Singhal, R. S., & Kulkarni, P. R. (2002). Quantification of blends of black gram and rice using pentosan as an indicator. *Food Chemistry*, *78*(1), 47–51.

Park, K. Y., Jeong, J. -K., Lee, Y. E., & Daily, J. W, 3rd (2014). Health benefits of kimchi (Korean fermented vegetables) as a probiotic food. *Journal of Medicinal Food*, *17*(1), 6–20.

Park, S., Ji, Y., Park, H., Lee, K., Park, H., Beck, B. R., et al. (2016). Evaluation of functional properties of lactobacilli isolated from Korean white kimchi. *Food Control*, *69*, 5–12.

Parvez, S., Malik, K. A., Ah Kang, S., & Kim, H. -Y. (2006). Probiotics and their fermented food products are beneficial for health. *Journal of Applied Microbiology*, *100*(6), 1171–1185.

Patra, J. K., Das, G., Paramithiotis, S., & Shin, H. -S. (2016). Kimchi and other widely consumed traditional fermented foods of Korea: A review. *Frontiers in Microbiology*, *7*, 1493.

Prajapati, J., & Nair, B. M. (2003). *History of fermented foods* (pp. 162–170). CRC Press.

QI Hai ping, Q. H. (2003). Natto—A promising food. *Chinese condiment* (pp. 11–14). Chinese condiment.

Ray, M., Ghosh, K., & Singh, S. (2016). Folk to functional: An explorative overview of rice-based fermented foods and beverages in India. *Journal of Ethnic Foods*, *3*(1), 5–18.

Ray, R. C., & Joshi, V. (2014). Fermented foods: Past, present and future. In *Microorganisms and fermentation of traditional foods* (pp. 1–36). Boca Raton, FL, USA: CRC Press.

Ricke, S. C., Diaz, I. Z., & Keeton, J. T. (2007). In *Fermented meat, poultry and fish products* (3rd ed., pp. 795–815). Washington, DC: American Society of Microbiology.

Ricke, S. C., Koo, O. K., & Keeton, J. T. (2013). Fermented meat, poultry, and fish products. In *Food microbiology* (pp. 857–880). Washington, DC: American Society of Microbiology.

Rossouw, D., & Bauer, F. F. (2016). Exploring the phenotypic space of non-*Saccharomyces* wine yeast biodiversity. *Food Microbiology*, *55*, 32–46.

Sarkar, P. K., Hasenack, B., & Nout, M. J. R. (2002). Diversity and functionality of *Bacillus* and related genera isolated from spontaneously fermented soybeans (Indian Kinema) and locust beans (African Soumbala). *International Journal of Food Microbiology*, *77*(3), 175–186.

Serrazanetti, D. I., Ndagijimana, M., & Miserocchi, C. (2013). Fermented tofu: Enhancement of keeping quality and sensorial properties. *Food Control*, *34*(2), 336–346.

Smit, G., Smit, B. A., & Engels, W. J. M. (2005). Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese products. *FEMS Microbiology Reviews*, *29*(3), 591–610.

Steinkraus, K. H. (1983). Indigenous fermented foods involving an acid fermentation/acid fermented cereal gruel. In *Handbook of indigenous fermented foods* (pp. 189–218). New York, NY: Mariel Dekkes, Inc..

Steinkraus, K. H. (1997). Classification of fermented foods: Worldwide review of household fermentation techniques. *Food Control*, *8*(5–6), 311–317.

Sumarmono, J., Sulistyowati, M., & Soenarto (2015). Fatty acids profiles of fresh milk, yogurt and concentrated yogurt from Peranakan Etawah goat milk. *Procedia Food Science*, *3*, 216–222.

Swain, M. R., Anandharaj, M., & Ray, R. C. (2014). Fermented fruits and vegetables of Asia: A potential source of probiotics. *Biotechnology Research International*, *2014*(2014), 250424.

Tamime, A., & Marshall, V. (1997). Microbiology and technology of fermented milks. In *Microbiology and biochemistry of cheese and fermented milk* (pp. 57–152). Boston, MA: Springer.

Thakur, K., Tomar, S. K., & De, S. (2016). Lactic acid bacteria as a cell factory for riboflavin production. *Microbial Biotechnology*, *9*(4), 441–451.

Torres, M. J., Brandan, C. P., Petroselli, G., Erra-Balsells, R., & Audisio, M. C. (2016). Antagonistic effects of *Bacillus subtilis* subsp. subtilis and *B. amyloliquefaciens* against *Macrophomina phaseolina*: SEM study of fungal changes and UV-MALDI-TOF MS analysis of their bioactive compounds. *Microbiological Research*, *182*, 31–39.

Vijayendra, S. V. N., & Halami, P. M. (2015). Health benefits of fermented vegetable products. *Health benefits of fermented foods and beverages* (pp. 325–342). Taylor & Francis.

Wang, H. L. (1984). Tofu and tempeh as potential protein sources in the western diet. *Journal of the American Oil Chemists' Society*, *61*(3), 528–534.

Weaver, K. E. (2015). The Type I toxin-antitoxin par locus from *Enterococcus faecalis* plasmid pAD1: RNA regulation by both cis- and trans-acting elements. *Plasmid*, *78*, 65–70.

Wu, TY, Tsai, CC, Hwang, YT, Chiu, TH, et al. (2012). Effect of antioxidant activity and functional properties of Chingshey purple sweet potato fermented milk by *Lactobacillus acidophilus*, *L. delbrueckii*subsp. lactis, and *L. gasseri* strains. *J Food Sci*, *77*(1), M2–M8.

Xu, L., Du, B., & Xu, B. (2015). A systematic, comparative study on the beneficial health components and antioxidant activities of commercially fermented soy products marketed in China. *Food Chemistry*, *174*, 202–213.

Chapter 25

Commercially Available Probiotics and Prebiotics Used in Human and Animal Nutrition

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1 Introduction

Both probiotics and prebiotics are considered as beneficial components used in human or animal nutrition having potential functional properties. Probiotics include nonpathogenic live microorganisms that when taken in sufficient amounts provide various health benefits to the host (Hill et al., 2014). Numerous studies have been conducted, which showed several advantageous properties and positive impact of probiotics on human and animal health. Presently, there is a growth in global market for probiotic products which is due to the increased demand for these products. The utilization of probiotics helps in the improvement of health and also prevents the host from various illnesses (Floch, 2014; Curro, Ianiro, Pecere, Bibbo, & Cammarota, 2017; Arora and Baldi, 2015). Modulation of intestinal microbiota is one of the proposed mechanisms for the activity of probiotics (Hemarajata & Versalovic, 2013). Nowadays, there are a number of bacterial strains available in the global probiotic market, which offer probiotic properties. Safety and efficacy of probiotics are considered the main factors involved in the formulation of probiotic products (Doron & Snydman, 2015). According to the regulations mentioned in food laws, probiotics should be safe for human and animal health. As per the regulations of the Food and Drug Administration (FDA) in the United States, microorganisms used as probiotics should possess generally regarded as safe (GRAS) status. In Europe the term of qualified presumption of safety (QPS) introduced by EFSA is used with regard to probiotic usage. The QPS concept includes some additional criteria for the safety of probiotics, namely, history of safe usage and the absence of the risk of acquired resistance to antibiotics (Anadón, Martínez-Larrañaga, & Martínez, 2006; Gaggia, Mattarelli, & Biavati, 2010). In addition to this, probiotic microorganisms should be able to survive during their transit through the gastrointestinal tract and on prolonged storage periods, large number of viable bacteria must be able to survive (Harish & Varghese, 2006). Several studies showed that probiotic microorganisms can be used singly or in combination with other microbial strains so as to act in a synergistic way for maintaining the health of the host (Khailova, Petrie, Baird, Dominguez Rieg, & Wischmeyer, 2014; Tham, Peh, Bhat, & Liong, 2012; Sarkar & Mandal, 2016). The microorganisms mostly used as probiotics in human nutrition are from the genera *Lactobacillus* and *Bifidobacterium*. Some yeast strains belonging to the genus *Saccharomyces* are also used in probiotic products (Simon, 2005). Probiotics used in animal nutrition mostly include species of *Lactobacillus*, *Bacillus*, *Bifidobacterium*, and *Streptococcus,* and some yeasts.

The term "prebiotics," introduced in 1995, refers to nondigestible substances that show beneficial effect on the host by helping in the selective stimulation of the growth and/or activity of beneficial microorganisms in the colon (Gibson & Roberfroid, 1995). Prebiotics act as a food for the microorganisms that are considered beneficial for health and well-being. Prebiotic substances undergo fermentation by beneficial microflora (probiotics) in the large intestine. A particular different prebiotics will enhance the growth of different indigenous gut microorganisms (Markowiak & Slizewska, 2017). Prebiotic substances should be resistant to digestion in the upper gastrointestinal tract and readily fermentable by potentially beneficial intestinal bacteria in the large intestine. The fermentation process should exert a positive effect on the host's health. In addition to this, prebiotics should help in selective stimulation of growth of the probiotic microorganism (Wang, 2009).

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00025-3
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TABLE 25.1 Examples of some commercially available probiotic bacterial strains used in human nutrition.

Strain	Source
Lactobacillus acidophilus NCFM	Rhodia, Inc. (Madison, WI, United States)
L. acidophilus LA-1 (or LA-5 in Europe)	Chr. Hansen, Inc. (Milwaukee, WI, United States)
L. acidophilus NCFB 1748	Arla (Stockholm, Sweden)
Lactobacillus paracasei Shirota (YIT 9018)	Yakult (Tokyo, Japan)
L. paracasei F19	Arla
Lactobacillus rhamnosus GG (ATCC 53103)	Valio Dairy (Helsinki, Finland)
L. rhamnosus 271	Probi AB
L. rhamnosus LB21	Essum AB (Umea, Sweden)
L. rhamnosus DR20 (HN001)	Fonterra Cooperative Group Ltd (Auckland, New Zealand)
Bifidobacterium longum BB536	Morinaga Milk Industry Co., Ltd. (Zama City, Japan)
Bifidobacterium breve strain Yakult	Yakult
Bifidobacterium lactis DR10 (HN019)	Fonterra Cooperative Group Ltd.

Source: Sanders, M. Y. (2003). *Nutrition Reviews*, *61*(3), 91–99.

Fruit, vegetables, cereals, and other edible plants are sources of carbohydrates that are included in potential prebiotics. Potential sources of prebiotics include tomatoes, artichokes, bananas, asparagus, berries, garlic, onions, chicory, green vegetables, legumes, as well as oats, linseed, barley, and wheat. Fructooligosaccharides, maltooligosaccharides, galactooligosaccharides (GOS), lactulose, cyclodextrins, and lactosucrose are some important artificially produced prebiotics (Crittenden & Playne, 2008).

2 Probiotic microorganisms used in human nutrition and their role

Promising probiotic bacterial strains used in human nutrition mostly include the members of genera *Lactobacillus* and *Bifidobacterium*. The important representative species include *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus gasseri, Lactobacillus rhamnosus, Bifidobacterium longum*, and *Bifidobacterium breve* (Fuller, 1991; Gordin & Gorbach, 1992). Other than bacteria also some yeast strains (*Saccharomyces cerevisiae* var. *boulardii*) are used as probiotics. Table 25.1 presents the various commercial probiotic microorganisms used in human nutrition (Tomasik & Tomasik, 2003).

2.1 *Lactobacillus* species

Lactobacillus refers to a group of lactic acid–producing Gram-positive rods that are obligate and facultative anaerobes in the human gastrointestinal and genitourinary tracts. The name *Lactobacillus* refers to the bacterium's ability to produce lactic acid, not to the ability to digest lactose. Lactobacilli are used therapeutically as probiotics, the opposite of antibiotics. They are considered "friendly" bacteria and are taken for the purpose of recolonizing areas of the body to provide nutritional benefits, including inducing growth factors and increasing the bioavailability of minerals. Lactobacilli also stabilize the mucosal barrier and decrease intestinal permeability. Taking *Lactobacillus* probiotics during antibiotic treatment can prevent or minimize normal flora depletion and pathogenic bacteria colonization. Hydrogen peroxide–producing lactobacilli are bactericidal to the vaginal pathogen *Gardnerella vaginalis*, and their presence in the vagina has been associated with decreased frequencies of bacterial vaginosis and trichomoniasis. Most researchers agree that the effectiveness of lactobacilli and other probiotics for all indications depends on their ability to colonize an area of tissue. To do this, *Lactobacillus* preparations must contain live and viable organisms. Lactobacilli strains might vary in their effectiveness due to differences in their ability to adhere to the epithelial cells by host factors such as hormone levels (Khalighi, Behdani, & Kouhestani, 2016). Some of the important commercially available human *Lactobacillus* probiotic strains are discussed in the following sections.

2.1.1 *Lactobacillus acidophilus LA-5*

Lactobacillus acidophilus LA-5, which was originally selected by Chr. Hansen for the development of probiotic dairy products, is being used worldwide in various dietary supplements and fermented dairy products. LA-5 is clinically well

documented and shows no undesirable effects on the taste, appearance, or palatability of the product. Furthermore, it has the potential to survive in the product until consumption. LA-5 has many probiotic features. It is able to survive passage through the stomach and upper small intestine due to its tolerance of stomach and bile acid and resistance to digestive enzymes (Noh & Gilliland, 1993). It is able to adhere to the intestinal mucosa (Juntunen, Kirjavainen, Ouwehand, Salminen, & Isolauri, 2001). *L. acidophilus* LA-5 belongs to the group of Gram-positive nonsporulating facultative or anaerobic rods, and it is a natural inhabitant of the gut. Lactobacilli play a significant role in controlling intestinal pH. Lactobacilli ferment glucose into lactic acid, acetic acid, and H_2O_2. These metabolites make the environment less favorable for the growth of potentially pathogenic microorganisms (Gorbach, 1990). LA-5 also produces bacteriocin CH5 that is characterized not only by a particularly wide antibacterial range (Chumchalova, Josephsen, & Plockova, 1995), but also by inhibitory action against certain yeasts and molds (Plockova, Tomanova, & Chumchalova, 1997). In patients with collagenous colitis and diarrhea, days per week with liquid feces were reduced from 6 to 1 in patients receiving LA-5 and BB-12 (Wildt et al., 2006). Administration of LA-5 has been associated with nonspecific stimulatory effects on the production of cytokines and phagocytic activity as well as more specific immune reactions such as antibody production. Health effects of LA-5 in humans seem to include inhibited growth of cancer breast cells (Biffi, Coradini, Larsen, Riva, & DiFronzo, 1997). In patients with acute leukemia, administration of LA-5 and BB-12 during and after chemotherapy significantly delayed the occurrence of fever, on average from day 8 in the placebo group to day 12 in the probiotic group (Ellegaard, Peterslund, & Black, 1992). In relation to the use of microorganisms as human probiotics, safety is clearly a major concern. Being a part of the normal human intestinal microbiota, Lactobacilli are generally considered safe, and millions of humans have consumed LA-5 without any reported negative side effects (Hoppe & Larsen, 2009).

2.1.2 *Lactobacillus acidophilus NCDO 1748*

The strain *L. acidophilus* NCDO 1748 (1748) was used in food products during 1970 due to its ability to grow in milk and its survival through the human gastrointestinal tract (Matto et al., 2006; 70, Pettersson, Graf, Alm, Lindwall, & Streomberg, 1983). In vitro and in vivo studies also showed that 1748 is resistant to low pH and/or bile acids. As per human studies, it has been predicted that strain 1748 showed favorable effect on the gut microbiota and gut function (Lidbeck, Gustafsson, & Nord, 1987). During antibiotic treatment, bacterial strain 1748 plays an important role in balancing the bacterial flora (Orrhage, Brismar, & Nord, 1994a; Orrhage, Sjostedt, & Nord, 2000). In vitro and in vivo studies have shown that strain 1748 binds and immobilizes food mutagens and thereby reducing their levels in vital internal organs like kidney and liver (Lidbeck et al., 1991; Lidbeck, Overvik, Rafter, & Nord, 1992; Orrhage, Sillerstrom, Gustafsson, Nord, & Rafter, 1994b; Orrhage, Annas, Nord, Brittebo, & Rafter, 2002). Furthermore, strain 1748 prevents intestinal epithelial cells from adverse effects of cytokines and also protects the cells from negative effects of enteroinvasive *Escherichia coli* (Resta-Lenert & Barett 2003; Resta-Lenert & Barett, 2006).

2.1.3 *Lactobacillus acidophilus NCFM*

Originally in the early 1970s, *L. acidophilus* North Carolina Food Microbiology (NCFM) was isolated from a human fecal sample. The strain derived its name NCFM from "North Carolina Food Microbiology," the research laboratory at North Carolina State University (NCSU) where isolation took place (Sanders & Klaenhammer, 2001). In vitro studies have shown revealed that the strain NCFM possesses various prerequisites to survive gastrointestinal tract, including resistance to bile juice, low pH, and digestive enzymes (Daniel et al., 2006). *L. acidophilus* NCFM also possesses antimicrobial activity as confirmed by the genome sequence of the strain (Dobson, Sanozky-Dawes, & Klaenhammer, 2007; Altermann et al., 2005). It has been helpful in reducing the lactose intolerance symptoms in case of children (Montes, Bayless, Saavedra, & Perman, 1995). Experimental studies have shown that when *L. acidophilus* NCFM is administered either singly or in combination with bacterial strains such as *Bifidobacterium lactis*, it helps in reducing the incidence of symptoms associated with common respiratory tract infections such as fever, cough, and runny nose (Ouwehand, Carcano, Li, & Leyer, 2007). *L. acidophilus* NCFM also has the ability to modulate the immune system responses. Following oral vaccination, *L. acidophilus* NCFM showed immunomodulatory effects on serum immunoglobulins as observed in healthy adults (Paineau et al., 2008). This improvement of immune responses confirms the ability of *L. acidophilus* NCFM in the prevention of common respiratory infections (Ouwehand et al., 2007). *L. acidophilus* NCFM is helpful in the formulation of synbiotic functional foods because of its ability to utilize fructooligosaccharides (Barrangou, Altermann, Hutkins, Cano, & Klaenhammer, 2003).

2.1.4 *Lactobacillus casei Shirota*

L. casei strain Shirota (LcS), which is used in the development of fermented probiotic milk drink, is clinically safe for consumption and possess potential health benefits as supported by various experimental studies. LcS has been used in

fermented milk products for more than 80 years and is one of the most intensively studied probiotics. Its benefits include improvement in the balance of intestinal microbes and volatile fatty acids in the gastrointestinal tract, antitumor activity, immune system activation, antimicrobial activity, and improvement in the frequency of defecation and stool quality (Asahara, Nomoto, Watanuki, & Yokokura, 2001; Matsuzaki, Takagi, Ikemura, Matsuguchi, & Yokokura, 2004; Nagao, Nakayama, Muto, & Okumura, 2000; Spanhaak, Havenaar, & Schaafsma, 1998; Matsumoto et al., 2010). Probiotic preparations containing LcS are helpful in prevent or treatment various types of diarrhea, including infectious diarrhea, traveler's diarrhea, and antibiotic-associated diarrhea. It may also be helpful in other digestive problems like colic constipation Crohn's disease, inflammatory bowel disease, irritable bowel syndrome, lactose intolerance, and ulcerative colitis (https://www.healthline.com/health/digestive-health/lactobacillus-casei#benefits).

2.1.5 *Lactobacillus gasseri OLL2716 (LG21)*

L. gasseri OLL2716 (LG21) is a prolific autochthonous microorganism that colonizes the gastrointestinal tract, oral cavity, and vagina in humans and has the ability to contribute potential probiotic activity. It belongs to group B acidophilus complex microorganism and can be differentiated from group A members by the way of genetic determination and the apparent absence of major surface-layer proteins (Pot et al., 1993; Boot, Kolen, Pot, Kersters, & Pouwels, 1996). LG21 shows high resistance to gastric juice, grows well under acidic conditions, and can be detected in the stomach of subjects who were orally given LG21. It is one of the most suitable bacterial strains to be used as an effective probiotic for *Helicobacter pylori* infection. In vitro studies depicted that LG21 produces large amounts of lactic acid that inhibits the growth of *H. pylori*. Lactic acid shows a strong inhibitory effect on the growth of *H. pylori* than hydrochloric acid at the same pH (Aiba, Suzuki, Kabir, Takagi, & Koga, 1998).

2.1.6 *Lactobacillus paracasei ssp. paracasei F19*

Lactobacillus paracasei ssp. paracasei F19 (F19) was first isolated from colonic mucosa of the healthy human. It has the ability to sustain in GI tract, various types of foods, and possess favorable food properties (232) and potential probiotic character (Ohlson, Bjorneholm, Fonden, & Svensson, 2002). It occurs naturally in foods and humans and has been isolated from cheese and several humans. F19 is being used in fermented milk products and various food products meant for children (Bjeorneholm, Ekleow, Saarela, & Meatteo, 2002; Crittenden et al., 2002; Fonden, Bjeorneholm, & Ohlson, 2003). F19 favorably influences the gut microbiota, and gut function of the healthy individuals as revealed by human studies (Sullivan, Palmgren, & Nord, 2001; Sullivan, Bennet, Viitanen, Palmgren, & Nord, 2002). F19 helps in the stabilization of gut microbiota, stool frequency, reducing the symptoms of irritable bowel syndrome, and in lowering the infection rate in case of immune-compromised patients and children. Oral administration of F19 to children during weaning helps in reducing the incidence of secondary infections thereby decreasing the need for antibiotic treatment, and in the case of women, it helps in reducing the symptoms of vaginosis (Eskesen & Larsen, 2009).

2.1.7 *Lactobacillus rhamnosus GG (LGG)*

L. rhamnosus strain GG (LGG), was originally isolated from fecal samples of a healthy human adult by Sherwood Gorbach and Barry Goldin; the GG is derived from their names explaining its typical surname letters GG. It possesses the necessary prerequisites meant for potential probiotic strain, including its resistance to stomach acid and bile, good growth characteristics, and good capacity to adhere with the intestinal epithelial layer (Doron, Snydman, & Gorbach, 2005). Both in vitro and in vivo studies have revealed that it has strong mucus adhering compared to other probiotic strains. It is one of the most widely studied probiotic strain that is used in number of commercially available probiotic products. The beneficial effects of LGG have been studied comprehensively in clinical trials and human studies. Various health benefits of LGG include the prevention and treatment of gastrointestinal infections and diarrhea and help in stimulation of immune system responses that promote vaccination or even prevent certain allergic symptoms (Segers & Lebeer, 2014).

2.1.8 *Lactobacillus rhamnosus GR-1*

This probiotic bacterial strain was originally isolated in 1980 from the distal urethra of a healthy woman. *L. rhamnosus* GR-1, a strain of commensal vaginal microbiota, has been investigated as a potential probiotic species. It is considered as a novel strain for vaginal probiotics in maintaining woman's health. *L. rhamnosus*, GR-1 has the ability to adhere well to urogenital epithelial and vaginal cells, and to temporarily colonize the human vagina and intestine following oral uptake. The strain has been documented to survive implantation on to the vaginal epithelium and interfere with urogenital pathogens (Petrova et al., 2016). The organism displaces and prevents adhesion by intestinal and urogenital pathogens, inhibits

the growth of harmful bacteria and yeast, can kill HIV and other viruses through its acid production, and stops pathogens from forming clumps or biofilms that are very difficult for drugs to eradicate. The strain also induces an antiinflammatory effect and modulates factors that would otherwise increase a woman's risk of preterm labor (Reid, inpress).

2.1.9 *Lactobacillus rhamnosus HN001*

L. rhamnosus HN001 (HN001) is a safe, immunity-stimulating probiotic with the ability to survive the adverse gastrointestinal conditions and the adherence to intestinal mucosa (Inturri, Stivala, & Blandino, 2015). This probiotic strain has strong immunomodulatory effect, improves human health, reduces the occurrence of eczema in children and inhibits the adhesion of pathogenic Gram-negative bacteria in intestinal environment (Cross, Mortensen, Kudsk, & Gill, 2002; Inturri, Stivala, Furneri, & Blandino, 2016). Humans studies revealed that the dietary consumption of low-fat milk or lactose-hydrolyzed low-fat milk supplemented with HN001 showed upregulation of systemic cellular immune responses in case of middle-aged and elderly persons (Sheih, Chiang, Wang, Liao, & Gill, 2001). In addition to this, supplementation with HN001 during pregnancy can effect immunity of fetus and immunomodulatory factors through breast milk (Prescott et al., 2008) and considerably reduces the cumulative frequency of eczema (Wickens et al., 2008).

2.1.10 *Lactobacillus reuteri RC-14*

Lactobacillus reuteri was first isolated in 1962 and is a well-studied probiotic bacterium that can colonize a large number of mammals. It is found in various parts of the human body sites, including the gastrointestinal tract, urinary tract, skin, and breast milk. It has been characterized as heterofermentative species that grows in oxygen-limited atmospheres and colonizes the GI tract of humans and animals (Kandler, Stetter, & Kohl, 1980). This organism can withstand a wide variety of pH environments, employs multiple mechanisms that allow it to successfully inhibit pathogenic microorganisms, and has been shown to secrete antimicrobial intermediaries. One species of *Lactobacillus, L. reuteri* has multiple beneficial effects on host health such as prevention and/or amelioration of diverse disorders. The fact that it normally colonizes the GI tract may be the reason it confers great probiotic properties (Jacobsen et al., 1999; Valeur, Engel, Carbajal, Connolly, & Ladefoged, 2004). It produces antimicrobial molecules, namely, organic acids, ethanol, which help in the inhibition of colonization of pathogenic microorganisms. *L. reuteri* also helps in the remodeling of commensal microbiota composition in the host. *L. reuteri* benefits the host immune system by decreasing the production of proinflammatory cytokines while promoting regulatory function and development of T cells. It has the ability to strengthen the intestinal barrier and reduce the microbial translocation from the gut lumen to the tissues which otherwise may cause various inflammatory diseases in the gut as well as in remote tissues. Decrease in the abundance of *L. reuteri* in humans has been correlated with an increase in the incidence of inflammatory diseases (Mu, Tavella, & Luo, 2018).

2.2 *Bifidobacterium* species

Bifidobacteria are Gram-positive, nonmotile, anaerobic bacteria and take a variety of shapes. These organisms are natural inhabitants in the gut of humans and animals and play a significant role in reducing intestinal pH by producing lactic and acetic acids, thereby restricting the growth of many potentially pathogenic bacteria (Hoppe & Larsen, 2009). Bifidobacteria, naturally present in the dominant colonic microbiota, represent up to 25% of the cultivable fecal bacteria in adults and 80% in infants. As probiotic agents, bifidobacteria have been studied for their efficacy in the prevention and treatment of gastrointestinal disorders, such as colonic transit disorders, intestinal infections, and colonic adenomas and cancer. The traditional use of bifidobacteria in fermented dairy products and the GRAS (generally regarded as safe) status of certain strains attest to their safety. Bifidobacteria are able to prevent or alleviate infectious diarrhea through their effects on the immune system and resistance to colonization by pathogens. There is some experimental evidence that certain bifidobacteria may actually protect the host from carcinogenic activity of intestinal flora (Picard et al., 2005). Some of the important commercially available probiotic strains of *Bifidobacterium* ssp. are *B. lactis HN019*, *Bifidobacterium animalis* ssp. lactis, BB-12, *B. breve* strain Yakult, and *B. longum* BB536.

2.1.1 *Bifidobacterium lactis HN019*

B. lactis HN019 is a probiotic strain for which many health benefits have been established, primarily related to immune enhancement. In vitro studies have shown that *B. lactis* HN019 has the necessary prerequisites to survive gastrointestinal transit; they are resistant to bile, low pH, and digestive enzymes (Prasad, Gill, Smart, & Gopal, 1998). Furthermore, the strains have been shown to adhere to human epithelial cell lines (Gopal, Prasad, Smart, & Gill, 2001a). The strains have thoroughly been investigated for their safety and have not been found to contain clinically significant transmissible

antibiotic resistance genes (Zhou, Pillidge, Gopal, & Gill, 2005). *B. lactis* HNO19 is a multipurpose probiotic bacteria, which lives in the gut. It is a powerful strain that has been proven to fight tumor growths, improve digestion, and enhance immune system functions. It can help to reduce the frequency and severity of respiratory diseases. Additionally, it has been shown to increase vaccination immune response in infants of mothers supplemented with *B. lactis* during pregnancy. In other words, these infants become further protected by the vaccine more quickly than infants without *B. lactis* supplementation. This strain is also responsible for breaking down body waste and aiding in the absorption of vitamins and minerals in the gut. One of the more subtle benefits of *B. lactis* HNO19 is that it makes it more difficult for foreign materials to pass through the gut wall and into the rest of the body. By lowering the permeability, *B. lactis* is able to maintain a tighter leash on what foreign substances make it to the rest of the body and lower the risk of harmful conditions, such as NEC and autoimmune diseases. It has been shown to decrease the toxic effects of gluten on the intestinal lining, and it could be involved in the prevention of celiac disease. *B. lactis* has been shown to help with digestion of lactose in individuals that are lactose intolerant. It is also an integral factor in supporting overall digestion of sugars, fibers, and macronutrients (Waller et al., 2011). In addition to their health benefits, *B. lactis* HN019 has been found to have excellent stability in supplements, dairy, and nondairy products (Gopal et al., 2005) and provide good flavor to fermented products. Furthermore, the strains are capable of utilizing GOS, providing options for formulating synbiotic functional foods (Gopal, Sullivan, & Smart, 2001b).

2.2.2 *Bifidobacterium animalis ssp. lactis BB-12*

B. animalis ssp. lactis, BB-12, was specially selected by Chr. Hansen for the production of probiotic dairy products. Technologically, BB-12 is expressing good stability and high acid and bile tolerance, also as freeze-dried products in dietary supplements. Furthermore, BB-12 has no adverse effects on taste, appearance, or palatability of the product and is also able to survive in the product until consumption (Hoppe & Larsen, 2009). BB-12 is a commercially available probiotic strain used throughout the world in a variety of functional foods and dietary supplements and its uses have been clinically demonstrated with both antiinflammatory and antipathogen properties. In the case of infants, incidence of diarrhea is minimized by using infant formulas supplemented with BB-12 (Saavedra, Bauman, Oung, Perman, & Yolken, 1994). Intake of fermented milk supplemented with BB-12 helps in normalization of bowel movements and reduction in the severity of lactose intolerance symptoms (Pitkealea et al., 2007). Administration of BB-12 has been associated with nonspecific stimulatory effects on the production of cytokines and phagocytic activity, as well as more specific immune reactions such as antibody production (Hoppe & Larsen, 2009).

2.2.3 *Bifidobacterium breve Yakult*

B. breve strain Yakult (BbrY) has been used in fermented milk products for many years and is one of the most intensively studied probiotics. Some of the various benefits of BbrY include improving the balance of intestinal microbes by increasing the numbers of beneficial bacteria and decreasing the numbers of harmful bacteria, increasing the total amount of volatile fatty acids in the gastrointestinal environment, decreasing urinary mutagenicity, activating the immune system and antiinfectious activity, and acting as an antiinflammatory bowel disease adjunct (Asahara et al., 1999; Imaoka and Umesaki 2009; Ishikawa et al., 2003; Kanamori et al., 2009; Sugawara et al., 2006).

2.2.4 *Bifidobacterium longum BB536*

BB536 was originally isolated from the gut of a healthy breast-fed infant in 1969. BB536 has been incorporated in various products such as milk-based drink, yogurt, infant formula, and nutritional supplements as functional food ingredient and marketed in over 30 countries for more than 40 years. In 1996 yogurt products containing BB536 have been awarded the Food for Special Health Uses (FOSHU) status by the Japanese Ministry of Health (He & Benno, 2010). BB536 is a Gram-positive, anaerobic, catalase-negative bacterium with irregular morphology. BB536 is highly accessible to human gut and highly stable in various finished products, including powdered formula, yogurt, and fermented milk with excellent stability during storage and high survivability in probiotic food until consumption (Abe, Miyauchi, Uchijima, Yaeshima, & Iwatsuki, 2009; Odamaki, Xiao, Yonezawa, Yaeshima, & Iwatsuki, 2011; Yonezawa et al., 2010). BB536 is well-evaluated for safety and has been listed on generally regarded as safe (GRAS) notice inventory (GRN No. 268) in the US FDA in 2009. The GRAS status was granted based on the evidence that BB536 is a nonpathogenic, nontoxigenic, nonhemolytic, and nonantibiotic-resistant probiotic bacterium that does not contain any plasmids and display harmful metabolic activities (FDA, 2009; Toscano, De Vecchi, Gabrieli, Zuccotti, & Drago, 2015; Xiao, Takahashi, Odamaki, Yaeshima, & Iwatsuki, 2010). *B. longum* subsp. *longum* BB536 is a clinically effective, well-established, multifunctional probiotic that has a long history of human use in alleviating gastrointestinal, immunological, and infectious diseases. *B. longum* subsp. *longum*

BB536 is one of the well-established probiotic strains with numerous profound health benefits in humans (Xiao, 2009). BB536 has long been recognized as one of the most effective probiotic strains for the improvement of gastrointestinal conditions. Mounting clinical evidences have shown that consumption of dairy products containing BB536, including yogurts, yogurt drinks, and nonfermented milks, can improve the frequency of defecation and fecal characteristics in healthy adults with constipation (Wong, Odamaki, & Xiao, 2019). BB536 has been reported to modulate gut motility and normalize defecation frequency in hospitalized elderly patients (Kondo et al., 2013). Supplementation of BB536 has been shown to confer health benefits by their immunomodulatory function and has been clinically evaluated for their application in the possible prevention and therapy of immune-mediated diseases. BB536 has also been shown to be effective for the treatment and control of microbial infections. Several in vitro and animal studies have demonstrated that BB536 can inhibit the growth of Gram-negative pathogens (Araya-Kojima, Yaeshima, Ishibashi, Shimamura, & Hayasawa, 1995; Inturri et al., 2016; Matsumoto et al., 2008). Competitive exclusion is one of the main reasons for the effectiveness of BB536 against pathogenic infections. BB536 interferes the adhesion of pathogenic bacteria to the epithelial cells (Inturri et al., 2016; Matsumoto et al., 2008). BB536 also may inhibit lethal activity of pathogenic bacteria via the release of metabolites such as lactic and acetic acids (Namba, Yaeshima, Ishibashi, Hayasawa, & Yamazaki, 2003).

2.3 Yeast

The yeasts constitute a large and heterogeneous group of microorganisms that are currently attracting increased attention from scientists and industry. Numerous and diverse biological activities make them promising candidates for a wide range of applications not limited to the food sector. In addition to their major contribution to flavor development in fermented foods, their antagonistic activities toward undesirable bacteria and fungi are now widely known. Yeasts are used in the preparation of human foods and beverages, where they, besides having technological functions, confer different beneficial effects on human health and well-being. Among these, the most well known is the probiotic effect, which has been proven for *S. cerevisiae* var. *boulardii*. *Saccharomyces boulardii*, isolated from litchi fruit in Indochina by Henri Boulard in the 1920s, is commonly used as probiotic yeast especially in the pharmaceutical industry. *S. boulardii* possesses many properties that make it a potential probiotic agent, that is, it survives transit through the GI tract and its optimum temperature is 37°C in vitro and in vivo, and it inhibits the growth of a number of microbial pathogens. *S. boulardii,* a patented yeast preparation, is the only yeast probiotic that has been proven effective in double-blind studies (Moslehi-Jenabian, Pedersen, & Jespersen, 2010). This is the only yeast produced and used as a pharmaceutical product offering numerous valuable effects such as prevention and treatment of intestinal diseases and immunomodulatory effects. This yeast is used in many countries as both a preventive and therapeutic agent for diarrhea and other gastrointestinal disorders caused by the administration of antimicrobial agents (Czerucka, Piche, & Rampal, 2007; Hatoum, Labrie, & Fliss, 2012).

3 Probiotic microorganisms used in animal nutrition and their role

The use of probiotics in animal feeds is popular because of its beneficial effects on health and stimulation of growth. Such formulations may contain one or more microorganic strains and may be administered as powder, suspension, tablet/capsule, pellet, or gel or paste, depending on the species and age of the host animals. They are being used regularly or constantly as feeding and prepremixing additives. The term "probiotics" is mostly applied to a group of bacteria belonging to genus *Lactobacillus*. Probiotic forms being used as feed additives include bacterial strains of lactic acid bacteria (LABs), *Lactobacillus* (*L. bulgaricus*, *L. acidophilus, L. casei, L. farciminis, L. plantarum, L. rhamnosus, L. lactis, L. salivarius*), *Bacillus* (*B. cereus* var. *toyoi, B. licheniformis*, *B. subtilis*), *Enterococcus* (*E. faecium, E. faecalis*), *Pediococcus* (*P. acidilactici*), *Streptococcus* (*S. infantarius, S. thermophilus*), *Bifidobacterium* spp., and particular strains of *E. coli* (Parvez, Malik, Ah Kang, & Kim, 2006). All of the microorganisms listed previously, except *L. Bulgaricus* and *S. thermophilus*, form natural components of intestinal flora, which are starter yogurt cultures. Besides this, bacteria, yeast, and fungal strains of *S. cerevisiae* and *Kluyveromyces* are also used as probiotics (Anadón et al., 2006). Bacteria such as *Lactobacillus* and *Enterococcus* are part of the natural microbiota of animals typically present at concentrations of 107–108 and 105–106 CFU g^{-1}. Generally, the gastrointestinal system does not contain yeast or bacillus bacteria. Specific microorganisms are preferred depending on the species and type of animal. Most of these microorganisms should potentially be safe for the host, as some of them may pose problems, for example, bacteria from enterococcus genes may be involved in antibiotic resistance transmission, and endotoxins and emetic toxins may be generated by the *B. cereus* strain. Besides this, the probiotic species must be present in adequate quantities. The recommended dosage for most probiotic strains is 109 CFU kg^{-1} (Simon, 2005). Probiotic microorganisms found in animal feed supplements are listed in Table 25.2. The various probiotics used in animal nutrition are discussed in the following sections.

TABLE 25.2 List of microorganisms intended mostly for animals (Bernardeau & Vernoux, 2013).

Type of *Lactobacillus*	Type of *Bifidobacterium*	Other lactic acid bacteria	Other microorganisms
L. brevis[a]	*B. animalis*[a]	*Enterococcus faecalis*	*Bacillus cereus*
L. casei[a]	*B. longum*[a]	*Enterococcus faecium*	*Bacillus licheniformis*[a]
L. crispatus[a]	*B. pseudolongum*	*Lactococcus lactis*[a]	*Bacillus subtilis*[a]
L. farciminis[a]	*B. thermophilum*	*Leuconostoc citreum*[a]	*Propionibacterium Freudenreichii*[a]
L. fermentum[a]		*Leuconostoc lactis*[a]	*Saccharomyces cerevisiae (boulardi)*[a]
L. murinus		*Leuconostoc mesenteroides*[a]	*Saccharomyces pastorianus*[a]
L. gallinarum[a]		*Pediococcus acidilactici*[a]	*Kluyveromyces fragilis*
L. paracasei[a]		*Pediococcus pentosaceus*[a]	*Kluyveromyces marxianus*[a]
L. pentosus[a]		*Streptococcus infantarius*	*Aspergillus oryzae*
L. plantarum[a]		*Streptococcus salivarius*	*Aspergillus niger*
L. reuteri[a]		*Streptococcus thermophilus*[a]	
L. rhamnosus[a]		*Sporolactobacillus inulinus*	
L. salivarius[a]			

[a]*QPS (qualified presumption of safety) microorganisms.*

3.1 Lactobacillus

Generally, LABs are nonspore-forming, microaerophilic, Gram-positive, catalase-negative microorganisms. The *Lactobacillus* genus comprises over 100 different species from the lactic acid production bacterium (LAB) group, a small and Heterogeneous Taxonomic Group. Many species of *Lactobacillus* are considered as components for human and animal gut microbiota, depending on the host, occurrence, and number. In the food chain, many LAB species participate in a broad range of fermentation in feed and nourishment and are used in probiotics for humans and animals (Markowiak & Śliżewska, 2018). Nevertheless, various studies indicated that these microorganisms are seldom responsible for human diseases where *L. casei* and *L. rhamnosus* are the most common (Hammes & Hertel, 2006). There is no report on animal safety concerns relating to lactobacilli. A list of species has been proposed for QPS status due to a long history of safe use (Vesterlund et al., 2007). The beneficial microbial strains of *Lactobacillus* used in animal feeds and having growth promotion effects are *L. acidophilus* (KNU 31), *Lactobacillus ingluviei* (CIP 102980), and L. ingluviei (CIP 102980) (Bernardeau & Vernoux, 2013). The probiotic potential of *Lactobacillus* has been reported due to its adhering ability to host intestinal cells. These have also been shown to produce toxic or antimicrobial substances European Food Safety Authority (EFSA), 2007] which includes organic acids, particularly lactic and acetic acids, bacteriocins such as nisin, a bacteriocin of *L. lactis* subsp. lactis and colicins produced by *E. coli* and hydrogen peroxide (Ouwehand, Isolauri, Kirjavainen, & Salminen, 1999). Several bacteriocins, including lactacin B, lactacin F, nisin, and reuterin, are developed by *Lactobacillus* probiotic strain (Nemcova, 1997). The ability to generate organic acids and bacteriocins is one of the mechanisms by which lactobacilli have a direct inhibitory effect on the growth of Gram-positive bacteria, food spoilage organisms, and pathogens (Wohlgemuth, Loh, & Blaut, 2010). These organic acids, together with probiotics and specific carbohydrates (yeast cell walls), are often suggested as alternatives to antibiotic growth promoters and are expected to be increasingly used as natural food preservatives (Piard & Desmazeaud, 1991; Jensen, 1998). LAB's immunomodulatory activity has been documented not only in innate immunity, such as regulation of Toll-like receptor activation of dendritic cells and natural killer cells, but also in adaptive immunity, including balancing T-helper (Th1/Th2) cell reactions and complex IgA secretion (Dykes, 1995). Also, *Lactobacillus* may make iron inaccessible for pathogenic microorganisms, either by binding ferric hydroxide to its surface or by chelating and transporting iron siderophores.

3.2 Bifidobacterium

Bifidobacteria are considered to be one of the primary generations of animals and the human intestinal tract. *B. animalis* subsp. *lactis* (503, DSM 16284) appears safe and has probiotic properties on the host (Tsai, Cheng, & Pan, 2012). Bifidobacteria are generally believed to maintain an appropriate microbiota balance in the GIT system, thus reduce the possibility of pathogenic infection (Holmes et al., 2012). Even though they are listed as species-specific and/or strain-specific probiotic

products, but they are very promising probiotics used in food and pharmaceutical preparations as well as for animal feedstuffs. Bifidobacteria may produce proteins or polypeptide bacteriocins that reduce the growth of closely related bacterial species (Biavati & Mattarelli, 2006; Yildirim, Winters, & Johnson, 1999). Basal diets complemented by a probiotic mixture containing *L. acidophilus, L. casei, E. faecium, and Bifidobacterium thermophilus* have been postulated to have increased IgG and IgM levels along with increased immunoglobulin levels in turkey, as increased immunoglobulin levels supported the growth and provide resistance to disease.

3.3 Bacillus

Bacillus is Gram-positive, endospore-forming aerobic facultative or anaerobic bacteria that generally occurs in soil, water, and air. The commonly used Bacillus strain species for animal nutrition are *B. subtilis* (KNU 42), *B. subtilis* (LS 1–2), *B. subtilis* (natto), and *B. licheniformis* (Bernardeau & Vernoux, 2013). CSIR (Biosciences, Pretoria, South Africa) developed a 6-string probiotic *Bacillus* spp. containing four *B. subtilis* (CPB 011, CPB 029, HP 1.6, and D 014) and two *Bacillus velezensis* (CBP 020 and CPB 035). The probiotic product is chosen based on its general performance in terms of animal probiotic characteristics such as proventriculus growth and survival pH, bile survival, extracellular enzyme (amylase, protease, cellulose, and xylanase), and adherence to epithelial cells. During production, birds are regularly challenged by a variety of stressors, including feed or pathogens. Through regulating intestinal microbiota, improvements help animals perform optimally even under challenging conditions and provide protection against future intestinal disorders. *Bacillus* bacteria in multiple-stranded preparations may affect potential growth stimulants for *Streptococcus* and *Lactobacillus* species (Cetin, Güçlü, & Cetin, 2005). Although *Bacillus* spp. do not adhere to the intestinal microvilli, they grow in the mucous biofilm over the intestinal villi, thus making the mucosa more suitable as a nutrient source for other probiotic bacteria (Pollmann, Danielson, Wren, Peo, & Shahani, 1980). In some clinical studies of pigs, bacillus species have also been used as probiotics. The inclusion of the strain of *B. subtilis* in the feed reduced the scores 24 h after the challenge of weaned pigs in the K88-positive ETEC (Porubcan, 1990). The supplementation of *B. licheniformis* and *B. subtilis* spores reduced morbidity and mortality in weaned piglets, enhancing the efficiency of fattening pigs and the quality of carcass (Bhandari, Xu, Nyachoti, Giesting, & Krause, 2008). However, the use of viable spores as a probiotic supplement has posed many concerns, particularly in terms of their safety, as various bacillus species are also recognized as food poisoning agents and have potential toxigenic characteristics (Alexopoulos et al., 2004). Based on the experimental findings, various studies revealed that *B. cereus* strains should be strongly discouraged (European Food Safety Authority (EFSA), 2007). Although, most of the bacillus species have potentially toxigenic characteristics, the toxigenic activity must be verified completely. Several studies revealed that their feed addition indicates that their safety could be ensured for at least certain species (Sanders et al., 2003). BioPlus 2B® probiotics containing *B. subtilis* and *B. licheniformis* strains improved the blood parameters of sows (higher cholesterol and lipid total) and milk parameters (higher milk fat and protein content) during milk feeding considerately. LSP 122 probiotic containing *B. licheniformis* spores was shown to be effective in regulating diarrhea syndrome in piglets 3–10 days after weaning (postweaned syndrome for diarrhea, PWDS) about clinical symptoms, mortality, weight gain, and feed conversion (Anadón et al., 2006). Infection with *E. coli* (ETEC) enterotoxigenic strains is the primary cause of morbidity and mortality of newborn piglets and recently weaned pigs. Overview of all the results of the study showed that the LSP 122 probiotic used at 107 viable spores of *B. licheniformis* is useful for combating PWDS caused by ETEC (Kritas, Govaris, Christodoulopoulos, & Burriel, 2006)

3.4 Saccharomyces

S accharomyces cerevisiae is a fermentative anaerobe commonly used in yeast culture. It is budding yeast that is also part of the residual intestinal microbiota system. The yeast culture contains compounds previously referred to as unidentified growth factor (UGF) that showed a positive effect on animal performance. *S. cerevisiae* is broadly distributed in the wild and is found in trees, fruit, and soil. *S. cerevisiae* (KNU 55) and *S. cerevisiae* KCTC No.7193 are used in animal products (Bernardeau & Vernoux, 2013). While *S. cerevisiae*-I 1079 is a specific live yeast strain designed for swine feeding, *S. boulardii* strain was isolated from the lychee skin and cultivated in Indochina. This species is not taxonomically classified and is considered a biotype of *S. cerevisiae* as well (Kyriakis et al., 1998). *S. boulardii*, is used as a probiotic, particularly in ruminants and pig feed. The probiotic mixture (*L. acidophilus* and *Saccharomyces cerevisiae*) shows beneficial effects on the intestinal microflora as they improved intestinal health by inhibiting pathogenic microorganisms such as bacterial *E. coli* and coliforms in lambs. A significant increase in milk production, along with an increase in milk immunoglobulin, lactoferrin, lysozyme, and lactoperoxidase content, has also been reported by (Xu et al., 2017) with a probiotic feed supplement containing Saccharomyces and Bacillus in dairy cows. On the other hand, there was a significant decrease in somatic cell counts with no significant difference in milk fat, protein, and lactose content. Bacterial probiotics are more common in

poultry, pigs, and young calves, whereas probiotic yeast strains (*S. cerevisiae*) and fungi (*Aspergillus oryzae*) are best used in adult ruminants (Yu et al., 1997).

Some of the other probiotic strains used in animal feed are *A. oryzae* KNU 48, *Clostridium butyricum*, *Propionibacterium freudenreichii* ssp. Shermanii CIRM-BIA129. The effect of steamed corn on their milk productivity was calculated by incorporating *A. oryzae* in cows' diet (Yu et al., 1997). The introduction of *A. oryzae* to steamed corn resulted in an increased percentage of protein and dry fat-free solids (Solids-Not-Fat, SNF) in milk. In recent years, the US-based company Bactana Animal Health is creating the new *Faecalibacterium prausnitzii* bacterial stain for commercial livestock use, in collaboration with the McGovern Institute for Life Sciences Venture Development at Cornell University. One of the companies involved in the development of pet probiotics, Microbial LLC (Gaithersburg, MD), has developed a multistrain, high-quality probiotic for companion dogs called Profauna 100. The product is specifically designed to promote canine digestive health. The list of certain companies involved in the respective probiotic microbial cultures for the health of their respective animals is listed in Table 25.3.

TABLE 25.3 Companies involved in probiotic microbial formulation as feed additive for targeted animal health (Markowiak & Śliżewska, 2018).

Trade name	Probiotic microbial cultures	Target animal
Yea-Sacc (Alltech)	*Lactobacillus rhamnosus, Enterococcus faecium*	Cattle, calves
Propoul (International Company s.r.o.)	*Lactobacillus fermentum*	Poultry
Probiosacc C-I	*Saccharomyces cerevisiae*	Calves
UltraCruz (Santa Cruz Animal Health)	*E. faecium, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus plantarum*	Cattle, calves, poultry
GalliPro® (Evonik Industries)	*Bacillus subtilis*	Poultry
Super-CyC (Choong Ang Biotech Co. Ltd.)	*B. subtilis, S. cerevisiae*	Poultry, cattle, horses, pigs
Protexin (Protexin Probiotics International Ltd.)	*L. plantarum, L. delbruecki* subsp., *Lactobacillus bulgaricus, Lactobacillus acidophilus, L. rhamnosus, Bifidobacterium bifidum; Streptococcus salivarius* subsp. *thermophilus; E. faecium; Aspergillus oryzae; Candida pintolepesii*	Poultry, pigs, sheep, cattle
Bro-biofair (Vitality Co.)	*Saccharomyces cerevisiae*	Pigs
Provita LE (Schaumann)	*L. rhamnosus, E. faecium*	Pigs, calves
Probios (Chr Hansen)	*Lactobacillus acidophilus, L. casei, L. plantarum, Lactobacillus lactis, E. faecium; B. subtilis*	Poultry, pigs
Acid-Pak-4-Way (Alltech)	*L. acidophilus, E. faecium*	Poultry, pigs
Probiomix	*B. bifidum, Lactobacillus amylovorus, E. faecium*	Calves, poultry
FloraMax-B11 (Pacific Vet Group)	*Lactobacillus salivarius, Pediococcus parvulus*	Poultry
Ecobiol (Norel Animal Nutrition)	*Bacillus amyloliquefaciens*	Poultry
Enviva™ Pro (DANISCO Animal Nutrition)	*B. subtilis*	Poultry
Cernivet LBC (Cerbios)	*E. faecium*	Calves, pigs
Cerbiogalli	*L. acidophilus, L. casei, L. plantarum*	Poultry
Cylactin (DSM)	*E. faecium*	Poultry, pigs, calves
Biogen T (Bio-Gen)	*B. bifidum, L. acidophilus, E. faecium*	Pigs
BioPlus2B® (Chr. Hansen)	*B. subtilis, Bacillus licheniformis*	Pigs, calves, poultry
BioPlus® YC (Evonik Industries)	*B. licheniformis, B. subtilis*	Pigs
B.I.O.Sol (Biochem)	*E. faecium*	Poultry
Calsporin (ORFFA)	*B. subtilis*	Poultry, pigs
Anta Pro EF (Dr. Eckel)	*E. faecium*	Pigs
Avian PAC (Soluble Loveland Industries)	*Streptococcus faecium, L. acidophilus*	Poultry
Biogen D (Bio-Gen)	*B. bifidum, L. acidophilus, Pediococcus faecium*	Poultry
Biogen N (Bio-Gen)	*B. bifidum, L. acidophilus, P. faecium*	Pigs

4 Prebiotics used in human and animal nutrition

Different sorts of microorganisms, known as gut microbiota, are occupants of the human gastrointestinal tract. It has been accounted that there are 1010–1012 live microorganisms for each gram in the human colon (Collins & Reid, 2016). The inhabitant microbial gatherings in the stomach and digestive organs are critical for human well-being. Most of these microorganisms, normally anaerobic, reside in the internal organ (Louis, Flint, & Michel, 2016). Albeit some endogenous variables, for example, mucin discharges can influence the microbial parity, the human eating regimen is the main wellspring of vitality for their development. Especially, nonedible sugars can exceptionally change the piece and capacity of gut microbiota (Walker et al., 2011). Gainful intestinal microorganisms age these nonedible dietary substances called prebiotics and acquire their endurance vitality from debasing unpalatable ties of prebiotics (Gibson & Roberfroid, 1995; Gibson, Probert, Van Loo, Rastall, & Roberfroid, 2004). Thus prebiotics can specifically impact gut microbiota (Bouhnik et al., 2004; Flint, Scott, Louis, & Duncan, 2012). Then again, gut microbiota influence intestinal capacities, for example, digestion and respectability of the digestive tract. Also, they may stifle pathogens in sound people by the enlistment of certain immunomodulatory atoms that have hostile impacts against pathogens by lactic corrosive that is delivered by *Bifidobacterium* and *Lactobacillus* genera (Morowvat, Nezafat, Ghasemi, Zare, & Mohkam, 2015; Shokri et al., 2018).

Prebiotic was described as "an ingredient of non-digestible food that has a beneficial effect on the host by selectively stimulating the growth and/or operation of one or a small number of bacteria in the colon, and thus improving host health. This definition was almost unchanged for more than 15 years. Only a few carbohydrate group compounds, such as short and long chain β-fructans [FOS and inulin], lactulose and GOS, can be categorized as prebiotics under this concept. The 6th meeting of the International Scientific Association of Probiotics and Prebiotics (ISAPP) in 2008 described 'dietary prebiotics' as 'a selectively fermented ingredient resulting in different modifications in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health" (Gibson et al., 2010). Commonly available prebiotics formualtions for animals and human are presented in Tables 25.4 and 25.5, respectively. There are several different kinds of prebiotics that find an important role in human and animal nutrition. The important ones include fructans, GOS, starch, glucose-derived oligosaccharides, and miscellaneous oligosaccharides.

4.1 Fructans

This class comprises inulin and fructooligosaccharide or oligofructose. Their structure is a straight chain of fructose with β(2→1) linkage. Inulin is composed of sucrose molecules in which fructose moiety is substituted with a linear chain of β(2→1) fructans ranging in length between 1 and 65 fructose moieties. Fructooligosaccharides are partially hydrolyzed inulin. Commercially available inulin is extracted from chicory root and fructans for animal nutrition contains more than 70% inulin, sugars, organic acids, protein fragments, and minerals. Fructan chains with a degree of polymerization (DP) < 10 are highly soluble, while as chains that have DP > 10 are slowly fermented. Inulin, as extracted from chicory roots, contains 30%–50% chains with DP < 10; while as rest are longer chains. However, oligofructose is composed of chains with DP < 10. The probiotic effect of inulin-type fructans in humans has been demonstrated in randomized, double-blind, and placebo-controlled studies in children, adults, and elderly and more recently in neonates. Given the well-defined probiotic effect of inulin-type fructans, these compounds are nowadays considered as the reference prebiotics and, therefore, frequently used in studies to compare the prebiotic effect of potential new compounds with. The inulin-type fructans are selectively fermented by bifidobacteria (and to a minor extend also lactobacilli). This colonic fermentation process

TABLE 25.4 List of available prebiotic formulations intended for livestock (Markowiak & Śliżewska, 2018).

Prebiotic preparation (producer)	Prebiotic substances	Destination
Bacto CS1000	Polysaccharides, oligosaccharides	Poultry
BionatStart	MOS, β-glucans	Calves
DOLSORB DN (Dolfos)	MOS, β-glucans	Poultry
MetSac MOS (VITTRA)	MOS, β-glucans	Calves, pigs, poultry
Mycocyd forte (Herbiline)	β-Glucans	Poultry
Mycostop (Extra-vit)	MOS, β-glucans	Poultry, pigs
PROFEED® (Beghin Meiji)	scFOS	Horses, pigs, poultry, calves

FOS, Fructooligosaccharides; *MOS*, malto-oligosaccharides; *scFOS*, short-chain fructooligosaccharides.

TABLE 25.5 Partial lists of commercial food products containing prebiotics that are widely available to consumers.

Company	Product	Claim	Inclusion level		Minimum daily dose (g/day)	Company web site
			g/Serving	Prebiotic type		
Horizon Organic, Boulder, CO	Various	Calcium absorption, immune benefits, improved digestion	0.750	Short-chain fructooligosaccharides	3	www.horizonorganic.com/
Stonyfield Farms, Londonderry, NH	Various	Calcium absorption	1.0–4.0	Short- and long-chain inulin	8	www.stonyfield.com
Lifeway Foods, Morton Grove, IL	Helios Nutrition's Organic Kefir	Bifidogenesis, calcium absorption	2	Short- and long-chain inulin	8	www.heliosnutrition.com
Kashi Company, La Jolla, CA	Cereals, drink mixes, cereal bars	Fiber	Check label	Short- and long-chain inulin listed as soluble fiber on Nutrition Facts label	8	www.kashi.com
Abbott Nutrition, Columbus, OH	Ensure fiber	Fiber, digestive health	1	Short-chain fructooligosaccharides	3	www.ensure.com
South Beach Diet, Miami Beach, FL	Cereal bars, meal replacement bars, and snacks	fiber	Varies	Oligofructose, inulin, or combination	8	www.southbeachdiet.com/kraft/kraft.asp
Clif Bar, Inc, Berkeley, CA	Clif Bar, Luna Bar, Builder's Bar	Fiber	Varies	Inulin, oligofructose	8	www.clifbar.com
Abbott Nutrition, Columbus, OH	ZonePerfect Shakes	Fiber	3	Fructooligosaccharides	3	www.zoneperfect.com
Skinny Cow, Nestle SA, Vevey, Switzerland	Low-fat ice cream sandwiches	Fiber	3	Fructooligosaccharides	3	www.skinnycow.com

generates organic acids (lactate and acetate) to which several beneficial effects linked to the ingestion of inulin-type fructans are (to some extent attributed) (Bosscher, 2009).

4.2 Galacto-oligosaccharides (GOS)

GOS are nondigestible and are derived from lactose composed of glucose α1–4 [β galactose 1–6]*n* and are commonly present in milk. GOS are ordered into two subgroups: (1) the GOS with overabundance galactose at C3, C4, or C6 and (2) the GOS made from lactose through enzymatic trans-glycosylation. Also, GOSs can significantly animate bifidobacteria and lactobacilli as this bifidogenic nature has been confirmed in rats (Holma, Juvonen, Asmawi, Vapaatalo, & Korpela, 2002) *Bacteroidetes* and *Firmicutes* are additionally invigorated by GOS, yet to a lesser degree than *Bifidobacteria* (Louis et al., 2016). Besides this, there are other GOS, types named raffinose family oligosaccharides.

4.3 Resistant starch (RS) and glucose-derived oligosaccharides

Another kind of prebiotic starch that is impervious to the upper gut absorption known as safe starch [resistant starch (RS)]. RS has been the subject of numerous studies that document a prebiotic effect, both as a single ingredient and in combination with fructooligosaccharides. These can advance well-being by producing significant level of butyrate (Fuentes-Zaragoza et al., 2011). Different gatherings of Firmicutes show the most elevated consolidation with a high measure of RS (Walker et al., 2011). An in vitro examination exhibited that RS could likewise be debased by *Ruminococcus bromii* and *Bifidobacterium adolescentis* furthermore less significantly by *Eubacterium rectale* and *Bacteroides thetaiotaomicron*.

In any case, in the blended bacterial and fecal hatching periods, RS corruption is unimaginable without *R. bromii* (Ze, Duncan, Louis, & Flint, 2012). Polydextrose is a glucose-determined oligosaccharide that comprises glucan with a great deal of glycosidic linkage branches. There is some proof that it can invigorate *Bifidobacteria*; however, it has not been affirmed at this point (Costabile et al., 2012). The benefit seen with fermentable and prebiotic dietary fibers is enhancement of mineral absorption and/or increase in bone mineralization and bone density. RS has been reported to enhance ileal absorption of a number of minerals in rats and humans. (Lopez et al., 2001) reported an increased absorption of calcium, magnesium, zinc, iron, and copper in rats fed RS2-rich diets. Similar preliminary results have been seen in an animal study investigating PROMITOR™ RS where it was shown to increase femur calcium concentration after 12 weeks of supplementation (Martin et al., 2008).

4.4 Miscellaneous oligosaccharides

A few oligosaccharides are begun from polysaccharide pectin and are called pectic oligosaccharide (POS). They depend on the expansion of galacturonic corrosive (homogalacturonan) or rhamnose (rhamnogalacturonan I). The carboxyl gatherings might be subbed with methyl esterification, and the structure can be acetylated at C2 or C3. Different sorts of sugars (e.g., arabinose, galactose, and xylose) or ferulic corrosive are connected to the side chains (Yoo, Kim, & Paek, 2012). Their structures fluctuate fundamentally relying upon the wellsprings of POSs (Gullón et al., 2013).

4.5 Lactulose

Lactulose is a synthetic derivative of lactose, consisting of one molecule of galactose and one molecule of fructose. It has traditionally been used as a laxative for treating human constipation (Tuohy, Rouzaud, Bruck, & Gibson, 2005). Lactulose is a prebiotic with a very specific application in dogs. It has been shown that small doses of the prebiotic act of lactulose are the growing number of human colonized bifidobacteria (Tuohy et al., 2002). When designing the composition of prebiotic formulas, it is important to determine a suitable dose. Supplementing canine diets with oligofructose or lactulose may lead to raised magnesium and calcium absorption, which can possibly be explained by an increased solubility of these minerals, caused by a lowered intestinal pH due to increased SCFA concentrations (Beynen, Kappert, & Yu, 2001).

4.6 Non-carbohydrates prebiotics

While carbohydrates are more likely to meet the prebiotics concept requirements, there are some compounds that are not categorized as carbohydrates but are recommended for classification as prebiotics, such as flavanols derived from cocoa. Experiments in vivo and in vitro demonstrate that flavanols can stimulate the bacteria from lactic acid (Tzounis et al., 2011).

5 Concluding remarks

Probiotics and prebiotics find a promising role for human and animal welfare. There is an increased demand for these safe natural ingredients to inhibit the proliferation of pathogenic bacteria and to modulate native microbiota of gastrointestinal tract so that the health condition, immune system, and performance of animals and humans could be improved. There is also a need to search for other prebiotic and probiotic options and introduce them in human and animal nutrition. Moreover, it has to be considered that pre- and probiotics are not a substitute for conventional medicine but act as supplements and adjuncts used in the prevention or treatment of diseases.

References

Abe, F., Miyauchi, H., Uchijima, A., Yaeshima, T., & Iwatsuki, K. (2009). Stability of bifidobacteria in powdered formula. *International Journal of Food Science & Technology*, *44*, 718–724. https://doi.org/10.1111/j.1365-2621.2008.01881.x.

Aiba, Y., Suzuki, N., Kabir, A. M., Takagi, A., & Koga, Y. (1998). Lactic acid-mediated suppression of *Helicobacter pylori* by the oral administration of *Lactobacillus salivarius* as a probiotic in a gnotobiotic murine model. *The American Journal of Gastroenterology*, *93*, 2097–2101.

Alexopoulos, C., Georgoulakis, I. E., Tzivara, A., Kritas, S. K., Siochu, A., & Kyriakis, S. C. (2004). Field evaluation of the efficacy of a probiotic containing *Bacillus licheniformis* and *Bacillus subtilis* spores, on the health status and performance of sows and their litters. *Journal of Animal Physiology and Animal Nutrition*, *88*(11–12), 381–392.

Altermann, E., Russell, W. M., Azcarate-Peril, M. A., Barrangou, R., Buck, B. L., & McAuliffe, O. (2005). Complete genome sequence of the probiotic lactic acid bacterium *Lactobacillus acidophilus* NCFM. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 3906–3912.

Anadón, A., Martínez-Larrañaga, M. R., & Martínez, M. A. (2006). Probiotics for animal nutrition in the European Union. Regulation and safety assessment. *Regulatory Toxicology and Pharmacology*, *45*(1), 91–95.

Araya-Kojima, T., Yaeshima, T., Ishibashi, N., Shimamura, S., & Hayasawa, H. (1995). Inhibitory effects of *Bifidobacterium longum*BB536 on harmful intestinal bacteria. *Bifidobacteria and Microflora*, *14*, 59–66. https://doi.org/10.12938/bifidus1982.14.2_59.

Arora, M., & Baldi, A. (2015). Regulatory categories of probiotics across the globe: A review representing existing and recommended categorization. *Indian Journal of Medical Microbiology*, *33*(Suppl.), 2–10. doi: 10.4103/0255-0857.150868.

Asahara, T., Nomoto, K., Watanuki, M., & Yokokura, T. (2001). Antimicrobial activity of intraurethrally administered probiotic *Lactobacillus casei* in a murine model of *Escherichia coli* urinary tract infection. *Antimicrobial Agents and Chemotherapy*, *45*, 1751–1760.

Asahara, T., Shimizu, K., Ohashi, Y., Matsuki, T., Matsumoto, K., Takada, T., et al. (1999). The effects of bifidobacteria-fermented milk on human urinary mutagenicity, which increases following ingestion of cooked ground beef. *Journal of Intestinal Microbiology*, *12*, 89–96.

Barrangou, R., Altermann, E., Hutkins, R., Cano, R., & Klaenhammer, T. R. (2003). Functional and comparative genomic analyses of an operon involved in fructooligosaccharide utilization by *Lactobacillus acidophilus*. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 8957–8962.

Bernardeau, M., & Vernoux, J. P. (2013). Overview of differences between microbial feed additives and probiotics for food regarding regulation, growth promotion effects and health properties and consequences for extrapolation of farm animal results to humans. *Clinical Microbiology and Infection*, *19*(4), 321–330.

Beynen, A., Kappert, H. J., & Yu, S. (2001). Dietary lactulose decreases apparent nitrogen excretion and increases apparent calcium and magnesium absorption in healthy dogs. *Journal of Animal Physiology and Animal Nutrition*, *85*, 67–72.

Bhandari, S. K., Xu, B., Nyachoti, C. M., Giesting, D. W., & Krause, D. O. (2008). Evaluation of alternatives to antibiotics using an *Escherichia coli* K88+ model of piglet diarrhea: Effects on gut microbial ecology. *Journal of Animal Science*, *86*(4), 836–847.

Biavati, B., & Mattarelli, P. (2006). The family Bifidobacteriaceae. In M. Dworkin, P. A. Hansen, & E. F. Lessel (Eds.), *The prokaryotes: Archaea. Bacteria: Firmicutes, actinomycetes* (pp. 322–382). (Vol. 3). New York, NY: Springer-Verlag.

Biffi, A., Coradini, D., Larsen, R., Riva, L., & DiFronzo, G. (1997). Antiproliferative effect of fermented milk on the growth of a human breast cancer cell line. *Nutrition and Cancer*, *28*, 93–99.

Bjeorneholm, S., Ekleow, A., Saarela, M., & Meatteo, J. (2002). Enumeration and identification of *Lactobacillus paracasei* ssp. paracasei F19. *Microbial Ecology in Health and Disease*, *3*, 7–13. Suppl. (Lactobacillus F19—Closing the broken circle).

Boot, H. J., Kolen, C. P., Pot, B., Kersters, K., & Pouwels, P. H. (1996). The presence of two S-layer-protein-encoding genes is conserved among species related to *Lactobacillus acidophilus*. *Microbiology*, *142*, 2375–2384.

Bosscher, D. (2009). Fructan prebiotics derived from inulin. In D. Charalampopoulos, & R. A. Rastall (Eds.), *Prebiotics and probiotics science and technology* (pp. 163–205). Springer Science & Business Media.

Bouhnik, Y., Raskine, L., Simoneau, G., Vicaut, E., Neut, C., Flourié, B., et al. (2004). The capacity of nondigestible carbohydrates to stimulate fecal bifidobacteria in healthy humans: A double-blind, randomized, placebo-controlled, parallel-group, dose-response relation study. *The American Journal of Clinical Nutrition*, *80*(6), 1658–1664.

Cetin, N., Güçlü, B. K., & Cetin, E. (2005). The effects of probiotic and mannanoligosaccharide on some haematological and immunological parameters in turkeys. *Journal of Veterinary Medicine Series A*, *52*(6), 263–267.

Chumchalova, J., Josephsen, J., & Plockova, M. (1995). Characterization of acidocin CH5, a saccharolytic sensitive bacteriocin of *Lactobacillus acidophilus* CH5. *Chemie, Mikrobiologie, Technologie der Lebensmittel*, *17*, 145–150.

Collins, S., & Reid, G. (2016). Distant site effects of ingested prebiotics. *Nutrients*, *8*(9), 523.

Costabile, A., Fava, F., Röytiö, H., Forssten, S. D., Olli, K., Klievink, J., et al. (2012). Impact of polydextrose on the faecal microbiota: A double-blind, crossover, placebo-controlled feeding study in healthy human subjects. *British Journal of Nutrition*, *108*(3), 471–481.

Crittenden, R., & Playne, M. J. (2008). Nutrition news: Facts and functions of prebiotics, probiotics and synbiotics. In Y. K. Lee, & S. Salminen (Eds.), *Handbook of probiotics and prebiotics* (pp. 535–582). Hoboken, NJ, USA; Manhattan, KS, USA: Wiley-Interscience, Kansas State University.

Crittenden, R., Saarela, M., Meatteo, J., Ouwehand, A. C., Salminen, S., Pelto, L., et al. (2002). *Lactobacillus paracasei* ssp. paracasei F19: Survival, ecology and safety in the human intestinal tract—A survey of feeding studies within the PROBDEMO project. *Microbial Ecology in Health and Disease*, *14*, 22–26.

Cross, M. L., Mortensen, R. R., Kudsk, J., & Gill, H. S. (2002). Dietary intake of *Lactobacillus rhamnosus* HNOO1 enhances production of both Th1 and Th2 cytokines in antigen-primed mice. *Medical Microbiology and Immunology*, *191*, 49–53.

Curro, D., Ianiro, G., Pecere, S., Bibbo, S., & Cammarota, G. (2017). Probiotics, fibre and herbal medicinal products for functional and inflammatory bowel disorders. *British Journal of Pharmacology*, *174*, 1426–1449. doi: 10.1111/bph.13632.

Czerucka, D., Piche, T., & Rampal, P. (2007). Review article: Yeast as probiotics – *Saccharomyces boulardii*. *Aliment Pharmacology Therapeutics*, *15*(26), 767–778. doi: 10.1111/j.1365-2036.2007.03442.x.

Daniel, C., Poiret, S., Goudercourt, D., Dennin, V., Leyer, G., & Pot, B. (2006). Selecting lactic acid bacteria for their safety and functionality by use of a mouse colitis model. *Applied and Environmental Microbiology*, *72*, 5799–5805.

Dobson, A. E., Sanozky-Dawes, R. B., & Klaenhammer, T. R. (2007). Identification of an operon and inducing peptide involved in the production of lactacin B by *Lactobacillus acidophilus*. *Journal of Applied Microbiology*, *103*, 1766–1778.

Doron, S., Snydman, D. R., & Gorbach, S. L. (2005). *Lactobacillus* GG: Bacteriology and clinical applications. *Gastroenterology Clinics*, *34*, 483–498.

Doron, S., & Snydman, D. R. (2015). Risk and safety of probiotics. *Clinical Infectious Diseases*, *60*(Suppl. 2), S129–S134. doi: 10.1093/cid/civ085.

Dykes, G. A. (1995). Bacteriocins: Ecological and evolutionary significance. *Trends in Ecology & Evolution*, *10*(5), 186–189.

Ellegaard, J., Peterslund, N. A., & Black, F. T. (1992). In *Infection prophylaxis in neutropenic patients by oral administration of Lactobacilli*Boulder, CO, June 21–24: The seventh international symposium on infections in the immunocompromised host.

Eskesen, D., & Larsen, C. N. (2009). *Lactobacillus paracasei* ssp paracasei, *L. casei* 431. In Y. K. Lee, & S. Salminen (Eds.), *Handbook of probiotics and prebiotics* (2nd ed., pp. 466–468). Hoboken, NJ: John Wiley & Sons, Inc..

European Food Safety Authority (EFSA). (2007). Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA-Opinion of the Scientific Committee. *EFSA Journal*, *5*(12), 587.

FDA. 2009. GRN No. 268. Retrieved July 10, 2018, from <http://wayback.archive-it.org/7993/20171031051412/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/UCM269214.pdf>

Flint, H. J., Scott, K. P., Louis, P., & Duncan, S. H. (2012). The role of the gut microbiota in nutrition and health. *Nature Reviews Gastroenterology & Hepatology*, *9*(10), 577.

Floch, M. H. (2014). Recommendations for probiotic use in humans—A 2014 update. *Pharmaceuticals (Basel)*, *7*, 999–1007. doi: 10.3390/ph7100999.

Fonden, R., Bjeorneholm, S., & Ohlson, K. (2003). Lactobacillus F19-safety considerations in practice. In *Fermented milk* (pp. 159–167). Brussels: IDF.

Fuentes-Zaragoza, E., Sánchez-Zapata, E., Sendra, E., Sayas, E., Navarro, C., Fernández-López, J., et al. (2011). Resistant starch as prebiotic: A review. *Starch-Stärke*, *63*(7), 406–415.

Fuller, R. (1991). Probiotics in human medicine. *Gut*, *32*, 439–442.

Gaggia, F., Mattarelli, P., & Biavati, B. (2010). Probiotics and prebiotics in animal feeding for safe food production. *International Journal of Food Microbiology*, *141*(Suppl.), 15–28.

Gibson, G. R., Probert, H. M., Van Loo, J., Rastall, R. A., & Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. *Nutrition Research Reviews*, *17*(2), 259–275.

Gibson, G. R., Scott, K. P., Rastall, R. A., Tuohy, K. M., Hotchkiss, A., Dubert-Ferrandon, A., et al. (2010). Dietary prebiotics: Current status and new definition. *Food Science & Technology Bulletin: Functional Foods*, *7*(1), 1–19.

Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *Journal of Nutrition*, *125*, 1401–1412.

Gopal, P., Dekker, J., Prasad, Y., Pillidge, C. J., Delabre, M. L., & Collett, M. (2005). Development and commercialisation of Fonterra's probiotic strains. *Australian Journal of Dairy Technology*, *60*, 173–182.

Gopal, P. K., Prasad, J., Smart, J., & Gill, H. S. (2001a). In vitro adherence properties of *Lactoacillus rhamnosus* DR20 and *Bifidobacterium lactis* DR10 strains and their antagonistic activity against an enterotoxigenic *Escherichia coli*. *International Journal of Food Microbiology*, *67*, 207–216.

Gopal, P. K., Sullivan, P. A., & Smart, J. B. (2001b). Utilisation of galacto-oligosaccharides as selective substrates for growth by lactic acid bacteria including *Bifidobacterium lactis* DR10 and *Lactobacillus rhmanosus* DR20. *International Dairy Journal*, *11*, 19–25.

Gorbach, S. L. (1990). Lactic acid bacteria and human health. Intestinal Microflora and Health. *Annals of Medicine*, *22*, 37–41.

Gordin, B. R., & Gorbach, S. L. (1992). Probiotics for humans. In R. Fuller (Ed.), *Probiotics. The scientific basis* (pp. 355–376). London: Chapman and Hall.

Gullón, B., Gómez, B., Martínez-Sabajanes, M., Yáñez, R., Parajó, J. C., & Alonso, J. L. (2013). Pectic oligosaccharides: Manufacture and functional properties. *Trends in Food Science and Technology*, *30*(2), 153–161.

Hammes, W. P., & Hertel, C. (2006). The genera *Lactobacillus* and *Carnobacterium*. *Prokaryotes*, *4*, 320–403.

Harish, K., & Varghese, T. (2006). Probiotics in humans – Evidence based review. *Calicut Medical Journal*, *4*, e3.

Hatoum, R., Labrie, S., & Fliss, I. (2012). Antimicrobial and probiotic properties of yeasts: From fundamental to novel applications. *Frontiers in Microbiology*, *3*, 421. doi: 10.3389/fmicb.2012.00421.

He, F., & Benno, Y. (2010). Probiotics and health claims: A Japanese perspective. In W. Kneifel, & S. Salmine (Eds.), *Probiotics and health claims* (pp. 118–125). Blackwell Publishing Ltd.: United Kingdom.

Hemarajata, P., & Versalovic, J. (2013). Effects of probiotics on gut microbiota: Mechanisms of intestinal immunomodulation and neuromodulation. *Therapeutic Advances in Gastroenterology*, *6*, 39–51. doi: 10.1177/1756283X12459294.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., et al. (2014). Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*, *11*, 506–514.

Holmes, E., Kinross, J., Gibson, G. R., Burcelin, R., Jia, W., Pettersson, S., et al. (2012). Therapeutic modulation of microbiota-host metabolic interactions. *Science Translational Medicine*, *4*(137), 137rv6.

Holma, R., Juvonen, P., Asmawi, M. Z., Vapaatalo, H., & Korpela, R. (2002). Galacto-oligosaccharides stimulate the growth of *bifidobacteria* but fail to attenuate inflammation in experimental colitis in rats. *Scandinavian Journal of Gastroenterology*, *37*(9), 1042–1047.

Hoppe, C., & Larsen, C. N. (2009). *Lactobacillus acidophilus*, LA-5. In Y. K. Lee, & S. Salminen (Eds.), *Handbook of probiotics and prebiotics* (2nd ed., pp. 441–444). Hoboken, NJ: John Wiley & Sons, Inc.. https://www.healthline.com/health/digestive-health/lactobacillus-casei#benefits.

Imaoka, A., & Umesaki, Y. (2009). Rationale for using *Bifidobacterium* probiotic strains-fermented milk against colitis based on animal experiments and clinical trials. *Probiotics and Antimicrobial Proteins*, *1*, 8–14.

Inturri, R., Stivala, A., Furneri, P. M., & Blandino, G. (2016). Growth and adhesion to HT-29 cells inhibition of Gram-negatives by *Bifidobacterium longum* BB536 e *Lactobacillus rhamnosus* HN001 alone and in combination. *European Review for Medical and Pharmacological Sciences*, *20*, 4943–4949.

Inturri, R., Stivala, A., & Blandino, G. (2015). Microbiological characteristics of the probiotic strains *B. longum* BB536 and *L. rhamnosus* HN001 used in combination. *Minerva Gastroenterologica e Dietologica*, *61*, 191–197.

Ishikawa, H., Akedo, I., Umesaki, Y., Tanaka, R., Imaoka, A., & Otani, T. (2003). Randomized controlled trial of the effect of bifidobacteria-fermented milk on ulcerative colitis. *Journal of the American College of Nutrition*, *22*, 56–63.

Jacobsen, C. N., Rosenfeldt, Nielsen V., Hayford, A. E., Moller, P. L., Michaelsen, K. F., Paerregaard, A., et al. (1999). Screening of probiotic activities of forty-seven strains of *Lactobacillus* spp. by in vitro techniques and evaluation of the colonization ability of five selected strains in humans. *Applied and Environmental Microbiology*, *65*, 4949–4956.

Jensen, B. B. (1998). The impact of feed additives on the microbial ecology of the gut in young pigs. *Journal of Animal and Feed Sciences*, *7*(Suppl. 1), 45–64.

Juntunen, M., Kirjavainen, P. V., Ouwehand, A. C., Salminen, S. J., & Isolauri, E. (2001). Adherence of probiotic bacteria to human intestinal mucus in healthy infants and during rotavirus infection. *Clinical and Vaccine Immunology*, *8*, 293–296.

Kanamori, Y., Iwanaka, T., Sugiyama, M., Komura, M., Takahashi, T., Yuki, N., et al. (2009). Early use of probiotics is an important therapy in infants with severe congenital anomaly. *Pediatrics International*, *52*, 362–367.

Kandler, O., Stetter, K. O., & Kohl, R. (1980). *Lactobacillus reuteri* sp. nov., a new species of heterofermentative lactobacilli. *Zentralblatt für Bakteriologie: I. Abt. Originale C: Allgemeine, angewandte und ökologische Mikrobiologie*, *1*, 264–269.

Khailova, L., Petrie, B., Baird, C. H., Dominguez Rieg, J. A., & Wischmeyer, P. E. (2014). *Lactobacillus rhamnosus* GG and *Bifidobacterium longum* attenuate lung injury and inflammatory response in experimental sepsis. *PLoS One*, *9*, e97861. doi: 10.1371/journal.pone.0097861.

Khalighi, A., Behdani, R., & Kouhestani, S. (2016). Probiotics: A comprehensive review of their classification, mode of action and role in human nutrition. *Probiotics and Prebiotics in Human Nutrition and Health*, *10*, 63646.

Kondo, J., Xiao, J., Shirahata, A., Baba, M., Abe, A., Ogawa, K., et al. (2013). Modulatory effects of *Bifidobacterium longum* BB536 on defecation in elderly patients receiving enteral feeding. *World Journal of Gastroenterology*, *19*, 2162–2170. https://doi.org/10.3748/wjg.v19.i14.2162.

Kritas, S. K., Govaris, A., Christodoulopoulos, G., & Burriel, A. R. (2006). Effect of *Bacillus licheniformis* and *Bacillus subtilis* supplementation of ewe's feed on sheep milk production and young lamb mortality. *Journal of Veterinary Medicine Series A*, *53*(4), 170–173.

Kyriakis, S. C., Sarris, K., Lekkas, S., Tsinas, A. C., Giannakopoulos, C., Alexopoulos, C., et al. (1998). In *Control of post weaning diarrhoea syndrome of piglets by in-feed application of origanum essential oils* (Vol. 218). Proceedings of the 15th IPVS congress.

Lidbeck, A., Geltner Allinger, U., Orrhage, K., Ottova, L., Brismar, B., Gustafsson, J. A., et al. (1991). Impact of *Lactobacillus acidophilus* supplements on the faecal microflora and soluble faecal bile acids in colon cancer patients. *Microbial Ecology in Health and Disease*, *4*, 81–88.

Lidbeck, A., Gustafsson, J. A., & Nord, C. E. (1987). Impact of *Lactobacillus acidophilus* supplements on human oropharyngeal and intestinal microflora. *Scandinavian Journal of Infectious Diseases*, *19*, 531–537.

Lidbeck, A., Overvik, J., Rafter, J., & Nord, C. E. (1992). Effect of *Lactobacillus acidophilus* supplements on mutagen excretion in faeces and urine in humans. *Microbial Ecology in Health and Disease*, *5*, 59–67.

Lopez, H. W., Levrat-Verny, M. A., Coudray, C., Besson, C., Krespine, V., Messager, A., & Remesy, C. (2001). Class 2 Resistant Starches Lower Plasma and Liver Lipids and Improve Mineral Retention in Rats. *The Journal of Nutrition*, *131*(4), 1283–1289.

Louis, P., Flint, H. J., & Michel, C. (2016). How to manipulate the microbiota: Prebiotics. In *Microbiota of the human body* (pp. 119–142). Cham: Springer.

Markowiak, P., & Slizewska, K. (2017). Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients*, *9*, 1021. doi: 10.3390/nu9091021.

Markowiak, P., & Śliżewska, K. (2018). The role of probiotics, prebiotics and synbiotics in animal nutrition. *Gut Pathogens*, *10*(1), 21.

Martin, F. P. J., Wang, Y., Sprenger, N., Yap, I. K., Lundstedt, T., Lek, P., et al. (2008). Probiotic modulation of symbiotic gut microbial–host metabolic interactions in a humanized microbiome mouse model. *Molecular Systems Biology*, *4*(1), 157.

Matsumoto, K., Takada, T., Shimizu, K., Moriyama, K., Kawakami, K., Hirano, K., et al. (2010). Effects of a probiotic fermented milk beverage containing *Lactobacillus casei* strain Shirota on defecation frequency, intestinal microbiota, and the intestinal environment of healthy individuals with soft stools. *Journal of Bioscience and Bioengineering*, *110*, 547–552.

Matsumoto, T., Ishikawa, H., Tateda, K., Yaeshima, T., Ishibashi, N., & Yamaguchi, K. (2008). Oral administration of *Bifidobacterium longum* prevents gut-derived *Pseudomonas aeruginosa* sepsis in mice. *Journal of Applied Microbiology*, *104*, 672–680. https://doi.org/10.1111/j.1365-2672.2007.03593.x.

Matsuzaki, T., Takagi, A., Ikemura, H., Matsuguchi, T., & Yokokura, T. (2004). Antitumor activity and action mechanisms of *Lactobacillus casei* through the regulation of immune responses. *Biofactors*, *22*, 63–66.

Matto, J., Fonden, R., Tolvanen, T., von Wright, A., Vilppon en-Salmela, T., Satokari, R., et al. (2006). Intestinal survival and persistence of probiotic *Lactobacillus* and *Bifidobacterium* strains administered in triple-strain yoghurt. *International Dairy Journal*, *16*, 1174–1180.

Montes, R. G., Bayless, T. M., Saavedra, J. M., & Perman, J. A. (1995). Effect of milks inoculated with *Lactobacillus acidophilus* or a yogurt starter culture in lactose-maldigesting children. *Journal of Dairy Science*, *78*, 1657–1664.

Morowvat, M. H., Nezafat, N., Ghasemi, Y., Zare, M. H., & Mohkam, M. (2015). Probiotic potential of five lactobacillus strains isolated from traditional Persian yoghurt in Fars province, Iran: Viewing through the window of phylogenetics. *Biosciences Biotechnology Research Asia*, *12*(2), 1265–1272.

Moslehi-Jenabian, S., Pedersen, L. L., & Jespersen, L. (2010). Beneficial effects of probiotic and food borne yeasts on human health. *Nutrients*, *2*, 449–473. doi: 10.3390/Nu2040449.

Mu, Q, Tavella, VJ, & Luo, XM (2018). Role of *Lactobacillus reuteri* in Human Health and Diseases. *Frontiers in Microbiology*, *9*, 757. doi: 10.3389/fmicb.2018.00757.

Nagao, F., Nakayama, M., Muto, T., & Okumura, K. (2000). Effects of a fermented milk drink containing *Lactobacillus casei* strain Shirota on the immune system in healthy human subjects. *Bioscience, Biotechnology, and Biochemistry*, *64*, 2706–2708.

Namba, K., Yaeshima, T., Ishibashi, N., Hayasawa, H., & Yamazaki, S. (2003). Inhibitory effects of *Bifidobacterium longum* on enterohemorrhagic *Escherichia coli* O157:H7. *Bioscience and Microflora*, *22*, 85–91. https://doi.org/10.12938/bifidus1996.22.85.

Nemcova, R. (1997). Selection criteria of lactobacilli for probiotic use. *Veterinarni Medicina*, *42*, 19–27.

Noh, D. O., & Gilliland, S. E. (1993). Influence of bile on cellular integrity and b-galactosidase activity of *Lactobacillus acidophilus*. *Journal of Dairy Science*, *76*, 1253–1259.

Odamaki, T., Xiao, J., Yonezawa, S., Yaeshima, T., & Iwatsuki, K. (2011). Improved viability of bifidobacteria in fermented milk by cocultivation with *Lactococcus lactis* subspecies lactis. *Journal of Dairy Science*, *94*, 1112–1121. https://doi.org/10.3168/jds.2010-3286.

Ohlson, K., Bjorneholm, S., Fonden, R., & Svensson, U. (2002). Lactobacillus F19 – A probiotic strain suitable for consumer products. *Microb. Ecol. Health Dis.*, *S3*, 27–32.

Orrhage, K., Annas, A., Nord, C. E., Brittebo, E. B., & Rafter, J. (2002). Effects of lactic acid bacteria on the uptake and distribution of the food mutagen Trp-P-2 in mice. *Scandinavian Journal of Gastroenterology*, *37*, 215–222.

Orrhage, K., Brismar, B., & Nord, C. E. (1994a). Effect of supplements with *Bifidobacterium longum* and *Lactobacillus acidophilus* on the intestinal microbiota during administration of clindamycin. *Microbial Ecology in Health and Disease*, *7*, 17–25.

Orrhage, K., Sillerstrom, E., Gustafsson, J. A., Nord, C. E., & Rafter, J. (1994b). Binding of mutagenic heterocyclic amines by intestinal and lactic acid bacteria. *Mutation Research*, *311*, 239–248.

Orrhage, K., Sjostedt, S., & Nord, C. E. (2000). Effect of supplements with lactic acid bacteria and oligofructose on the intestinal microflora during administration of cefpodoxime proxetil. *Journal of Antimicrobial Chemotherapy*, *46*, 603–612.

Ouwehand, A. C., Isolauri, E., Kirjavainen, P. V., & Salminen, S. J. (1999). Adhesion of four *Bifidobacterium* strains to human intestinal mucus from subjects in different age groups. *FEMS Microbiology Letters*, *172*(1), 61–64.

Ouwehand, A. C., Carcano, D., Li, S., & Leyer, G. (2007). Probiotics reduce incidence and duration of respiratory tract infection symptoms in 3–5-year-old children. *Cibus*, *3*, 60.

Paineau, D., Carcano, D., Leyer, G., Darquy, S., Alyanakian, M. A., Simoneau, G., et al. (2008). Effects of seven potential probiotic strains on specific immune responses in healthy adults: A double-blind, randomized, controlled trial. *FEMS Immunology and Medical Microbiology*, *53*, 107–113.

Parvez, S., Malik, K. A., Ah Kang, S., & Kim, H. Y. (2006). Probiotics and their fermented food products are beneficial for health. *Journal of Applied Microbiology*, *100*(6), 1171–1185.

Petrova, M. I., Lievens, E., Verhoeven, T. L., Macklaim, J. M., Gloor, G., Schols, D., et al. (2016). The lectin-like protein 1 in *Lactobacillus rhamnosus* GR-1 mediates tissue-specific adherence to vaginal epithelium and inhibits urogenital pathogens. *Scientific Reports*, *6*, 37437. https://doi.org/10.1038/srep37437.

Pettersson, L., Graf, W., Alm, L., Lindwall, S., & Streomberg, A. (1983). Survival of *Lactobacillus acidophilus* NCDO 1748 in the human gastrointestinal tract 1. Incubation in gastric juice. In B. Hallgren (Ed.), *Nutrition and the intestinal microflora* (pp. 123–126). Stockholm, Sweden: Almqvist & Wiksell International.

Piard, J. C., & Desmazeaud, M. (1991). Inhibiting factors produced by lactic acid bacteria. 1. Oxygen metabolites and catabolism end-products. *Le Lait*, *71*(5), 525–541.

Picard, C., Fioramonti, J., Francois, A., Robinson, T., Neant, F., & Matuchansky, C. (2005). Review article: Bifidobacteria as probiotic agents—Physiological effects and clinical benefits. *Alimentary Pharmacology & Therapeutics*, *22*, 495–512. doi: 10.1111/j.1365-2036.2005.02615.x.

Pitkealea, K. H., Strandberg, T. E., FinneSoveri, U. H., Ouwehand, A. C., Poussa, T., & Salminen, S. (2007). Fermented cereal with specific bifidobacteria normalizes bowel movements in elderly nursing home residents: A randomized, controlled trial. *The Journal of Nutrition, Health and Aging*, *11*, 305–311.

Plockova, M., Tomanova, J., & Chumchalova, J. (1997). Inhibition of mould growth and spore production by *Lactobacillus acidophilus* CH5 metabolites. *Bulletin of Food Research*, *36*, 237–247.

Pollmann, D. S., Danielson, D. M., Wren, W. B., Peo, E. R., Jr., & Shahani, K. M. (1980). Influence of *Lactobacillus acidophilus* inoculum on gnotobiotic and conventional pigs. *Journal of Animal Science*, *51*(3), 629–637.

Porubcan, R. S. (1990). Probiotics in the 1990s. *The Compendium on Continuing Education for the Practicing Veterinarian (USA)*, *12*, 1353–1359.

Pot, B., Hertel, C., Ludwig, W., Descheemaeker, P., Kersters, K., & Schleifer, K. H. (1993). Identification and classification of *Lactobacillus acidophilus*, *L. gasseri* and *L. johnsonii* strains by SDS-PAGE and rRNA-targeted oligonucleotide probe hybridization. *The Journal of General Microbiology*, *139*, 513–517.

Prasad, J., Gill, H., Smart, J., & Gopal, P. K. (1998). Selection and characterisation of *Lactobacillus* and *Bifidobacterium* strains for use as probiotics. *International Dairy Journal*, *8*, 993–1002.

Prescott, S. L., Wickens, K., Westcott, L., Jung, W., Currie, H., Black, P. N., et al. (2008). Supplementation with *Lactobacillus rhamnosus* or *Bifidobacterium lactis* probiotics in pregnancy increases cord blood interferon-γ and breast milk transforming growth factor-β and immunoglobulin A detection. *Clinical & Experimental Allergy*, *38*, 1606–1614.

Reid, G. *Probiotics for women's health. The story of Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14*. <https://www.jarrow.com/articles/release/id/403/Probiotics_for_Women's_Health:_The_Story_of_Lactobacillus_Rhamnosus_GR-1_and_Lactobacillus_Reuteri_RC-14>.

Resta-Lenert, S., & Barett, K. E. (2006). Probiotics and commensals reverse TNF-alpha- and IFN-gamma-induced dysfunction in human intestinal epithelial cells. *Gastroenterology*, *130*, 731–746.

Resta-Lenert, S., & Barett, K. E. (2003). Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive *Escherichia coli* (EIEC). *Gut*, *52*, 988–997.

Saavedra, J. M., Bauman, N. A., Oung, I., Perman, J. A., & Yolken, R. H. (1994). Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhea and shedding of rotavirus. *Lancet*, *344*, 1046–1049.

Sanders, M. E., Morelli, L., & Tompkins, T. A. (2003). Sporeformers as human probiotics: *Bacillus*, *Sporolactobacillus*, and *Brevibacillus*. *Comprehensive Reviews in Food Science and Food*, *2*(3), 101–110.

Sanders, M. E., & Klaenhammer, T. R. (2001). The scientific basis of *Lactobacillus acidophilus* NCFM functionality as a probiotic. *Journal of Dairy Science*, *84*, 319–331.

Sarkar, A., & Mandal, S. (2016). Bifidobacteria-insight into clinical outcomes and mechanisms of its probiotic action. *Microbiological Research*, *192*, 159–171. doi: 10.1016/j.micres.2016.07.001.

Segers, M. E., & Lebeer, S. (2014). Towards a better understanding of *Lactobacillus rhamnosus* GG – Host interactions. *Microbial Cell Factories*, *13*(Suppl. 1), S7. http://www.microbialcellfactories.com/content/13/S1/S7. From 11th International Symposium on Lactic Acid Bacteria Egmond aan Zee, the Netherlands. 31 August–4 September 2014.

Sheih, Y. H., Chiang, B. L., Wang, L. H., Liao, C. K., & Gill, H. S. (2001). Systemic immunity-enhancing effects in healthy subjects following dietary consumption of the lactic acid bacterium *Lactobacillus rhamnosus* HN001. *Journal of the American College of Nutrition*, *20*, 149–156.

Shokri, D., Khorasgani, M. R., Mohkam, M., Fatemi, S. M., Ghasemi, Y., & Taheri-Kafrani, A. (2018). The inhibition effect of lactobacilli against growth and biofilm formation of *Pseudomonas aeruginosa*. *Probiotics and Antimicrobial Proteins*, *10*(1), 34–42.

Simon, O. (2005). Micro-organisms as feed additives—Probiotics. *Advances in Pork Production*, *16*, 161–167.

Spanhaak, S., Havenaar, R., & Schaafsma, G. (1998). The effect of consumption of milk fermented by *Lactobacillus casei* strain Shirota on the intestinal microflora and immune parameters in humans. *European Journal of Clinical Nutrition*, *52*, 899–907.

Sugawara, G., Nagino, M., Nishio, H., Ebata, T., Takagi, K., Asahara, T., et al. (2006). Perioperative synbiotic treatment to prevent postoperative infectious complications in biliary cancer surgery: A randomized controlled trial. *Annals of Surgery*, *244*, 706–714.

Sullivan, A., Bennet, R., Viitanen, M., Palmgren, A. C., & Nord, C. E. (2002). Influence of *Lactobacillus* F19 on intestinal microflora in children and elderly persons and impact on *Helicobacter pylori* infections. *Microbial Ecology in Health and Disease*, *3*, 17–21. Suppl. (Lactobacillus F19—Closing the broken circle).

Sullivan, A., Palmgren, A. C., & Nord, C. E. (2001). Effect of *Lactobacillus paracasei* on intestinal colonisation of *Lactobacilli*, *Bifidobacteria* and *Clostridium difficile* in elderly persons. *Anerobe*, *07*, 67–70.

Tham, C. S., Peh, K. K., Bhat, R., & Liong, M. T. (2012). Probiotic properties of bifidobacteria and lactobacilli isolated from local dairy products. *Annals of Microbiology*, *62*, 1079–1087. doi: 10.1007/s13213-011-0349-8.

Tomasik, P. J., & Tomasik, P. (2003). Probiotics and prebiotics. *Cereal Chemistry*, *80*(2), 113–117.

Toscano, M., De Vecchi, E., Gabrieli, A., Zuccotti, G. V., & Drago, L. (2015). Probiotic characteristics and in vitro compatibility of a combination of *Bifidobacterium breve* M-16V, *Bifidobacterium longum* subsp. infantis M-63 and *Bifidobacterium longum* subsp. longum BB536. *Annals of Microbiology*, *65*(2), 1079–1086. https://doi.org/10.1007/s1321.

Tsai, Y. T., Cheng, P. C., & Pan, T. M. (2012). The immunomodulatory effects of lactic acid bacteria for improving immune functions and benefits. *Applied Microbiology and Biotechnology*, *96*(4), 853–862.

Tuohy, K. M., Rouzaud, G. C. M., Bruck, W. M., & Gibson, G. R. (2005). Modulation of the human gut microflora towards improved health using prebiotics-assessment of efficacy. *Current Pharmaceutical Design*, *11*(1), 75–90.

Tuohy, K. M., Ziemer, C. J., Klinder, A., Knöbel, Y., Pool-Zobel, B. L., & Gibson, G. R. (2002). A human volunteer study to determine the prebiotic effects of lactulose powder on human colonic microbiota. *Microbial Ecology in Health and Disease*, *14*(3), 165–173.

Tzounis, X., Rodriguez-Mateos, A., Vulevic, J., Gibson, G. R., Kwik-Uribe, C., & Spencer, J. P. (2011). Prebiotic evaluation of cocoa-derived flavanols in healthy humans by using a randomized, controlled, double-blind, crossover intervention study. *The American Journal of Clinical Nutrition*, *93*(1), 62–72.

Valeur, N., Engel, P., Carbajal, N., Connolly, E., & Ladefoged, K. (2004). Colonization and immunomodulation by *Lactobacillus reuteri* ATCC 55730 in the human gastrointestinal tract. *Applied and Environmental Microbiology*, *70*, 1176–1181.

Vesterlund, S., Vankerckhoven, V., Saxelin, M., Goossens, H., Salminen, S., & Ouwehand, A. C. (2007). Safety assessment of *Lactobacillus* strains: Presence of putative risk factors in faecal, blood and probiotic isolates. *International Journal of Food Microbiology*, *116*(3), 325–331.

Walker, A. W., Ince, J., Duncan, S. H., Webster, L. M., Holtrop, G., Ze, X., et al. (2011). Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *The ISME Journal*, *5*(2), 220–230.

Waller, P. A., Gopal, P. K., Leyer, G. J., Ouwehand, A. C., Reifer, C., Stewart, M. E., et al. (2011). Dose-response effect of *Bifidobacterium lactis* HN019 on whole gut transit time and functional gastrointestinal symptoms in adults. *Scandinavian Journal of Gastroenterology*, *46*, 1057–1064. doi: 10.3109/00365521.2011.584895.

Wang, Y. (2009). Prebiotics: Present and future in food science and technology. *Food Research International*, *42*, 8–12.

Wickens, K., Black, P. N., Stanley, T. V., Mitchell, E., Fitzharris, P., Tannock, G. W., et al. (2008). A differential effect of 2 probiotics in the prevention of eczema and atopy: A double-blind, randomized, placebo-controlled trial. *The Journal of Allergy and Clinical Immunology*, *122*, 788–794.

Wildt, S., Munck, L. K., Vinter-Jensen, L., Hansen, B. F., Nordgaard-Lassen, I., Christensen, S., et al. (2006). Probiotic treatment of collagenous colitis: A randomized, double-blind, placebo-controlled trial with *L. acidophilus* and *Bifidobacterium animalis* subsp. lactis. *Inflammatory Bowel Diseases*, *12*, 395–401.

Wohlgemuth, S., Loh, G., & Blaut, M. (2010). Recent developments and perspectives in the investigation of probiotic effects. *International Journal of Medical Microbiology Supplements*, *300*(1), 3–10.

Wong, C. B., Odamaki, T., & Xiao, J. -Z. (2019). Beneficial effects of *Bifidobacterium longum* subsp. longum BB536 on human health: Modulation of gut microbiome as the principal action. *Journal of Functional Foods*, *54*, 506–519.

Xiao, J. (2009). Probiotic *Bifidobacterium longum* BB536. In Y. K. Lee, & S. Salminen (Eds.), *Handbook of probiotics and prebiotics* (pp. 488–491). Hoboken, NJ: John Wiley & Sons Inc..

Xiao, J., Takahashi, S., Odamaki, T., Yaeshima, T., & Iwatsuki, K. (2010). Antibiotic susceptibility of bifidobacterial strains distributed in the Japanese market. *Bioscience, Biotechnology, and Biochemistry*, *74*, 336–342.

Xu, H., Huang, W., Hou, Q., Kwok, L., Sun, Z., Ma, H., & Zhang, H. (2017). The effects of probiotics administration on the milk production, milk components and fecal bacteria microbiota of dairy cows. *Science Bulletin*, *62*(11), 767–774.

Yildirim, Z., Winters, D. K., & Johnson, M. G. (1999). Purification, amino acid sequence and mode of action of bifidocin B produced by *Bifidobacterium bifidum* NCFB 1454. *Journal of Applied Microbiology*, *86*(1), 45–54.

Yonezawa, S., Xiao, J., Odamaki, T., Ishida, T., Miyaji, K., Yamada, A., et al. (2010). Improved growth of bifidobacteria by cocultivation with *Lactococcus lactis* subspecies lactis. *Journal of Dairy Science*, *93*, 1815–1823. https://doi.org/10.3168/jds.2009-2708.

Yoo, H. D., Kim, D., & Paek, S. H. (2012). Plant cell wall polysaccharides as potential resources for the development of novel prebiotics. *Biomolecules & Therapeutics*, *20*(4), 371.

Yu, P., Huber, J. T., Theurer, C. B., Chen, K. H., Nussio, L. G., & Wu, Z. (1997). Effect of steam-flaked or steam-rolled corn with or without *Aspergillus oryzae* in the diet on performance of dairy cows fed during hot weather. *International Journal of Dairy Science*, *80*(12), 3293–3297.

Ze, X., Duncan, S. H., Louis, P., & Flint, H. J. (2012). *Ruminococcus bromii* is a keystone species for the degradation of resistant starch in the human colon. *The ISME Journal*, *6*(8), 1535–1543.

Zhou, J. S., Pillidge, C. J., Gopal, P. K., & Gill, H. S. (2005). Antibiotic susceptibility profiles of new probiotic *Lactobacillus* and *Bifidobacterium* strains. *International Journal of Food Microbiology*, *98*, 211–217.

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Chapter 26

New Formulations and Products in Prebiotic Food

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1 Introduction

Prebiotics are food components that selectively stimulate the growth and activity of probiotics. They are nondigestible food ingredients consisting of short-chain carbohydrates, principally oligosaccharides such as fructooligosaccharides (FOSs), galactooligosaccharides (GOSs), and inulin (Gibson, 1995; Panitantum, 2004). They are not digested or absorbed in the early part of the digestive tract but can be hydrolyzed by probiotic bacteria in the large intestine, converting them to compounds beneficial to the host's health. Prebiotics are more useful as functional food than probiotics since they can be added to more foods than probiotics, because of their ability to survive the digestive process in the upper gastrointestinal (GI) tract (Vernazza, Rabiu, & Gibson, 2006). Oligosaccharides, such as GOSs, and FOSs, are used extensively as ingredients in many food products. Most of these ingredients currently available in the market are synthesized. These compounds may also be found naturally in chicory, onion, artichoke, asparagus, banana, garlic, soybean, and others, and their functionality may be enhanced further through biotechnological approaches (Gibson, Ottaway, & Rastall, 2000).

Production of functional foods containing prebiotic ingredients is an area that has dominant featuring in the food industry in recent years and a very promising market, not only for economic reasons but also by scientific evidence of its benefits. Consumers are more aware of the relationship between good nutrition and increasing seek for food that in addition to nurture provide health benefits (Burgain, Gaiani, Linder, & Scher, 2011). Aiming to satisfy this new market, some prebiotics, especially inulin and FOS, have been incorporated into a wide variety of foods and beverages that are part of a natural diet like dairy products, bread, cereals, and dietary supplements. Through this growth, consumers can appreciate tasty meals while promoting beneficial effects on their own health (Coman et al., 2012). Prebiotics can be formulated either as a powder or syrup and marketed as supplements or incorporated into food products.

Nowadays, consumers are becoming more health-conscious and concerned about the beneficial value of food, thus directing manufacturers to emphasize the promotion of functional foods. The change of consumer preferences, needs, and acceptances is a dynamic process; hence, the maintenance of food quality via technology innovation is evident. These compounds when administered in defined quantitative and qualitative amounts will provide clinically proven health benefits apart from those delivered by fundamental nutrients. The development of prebiotic food formulations is a key research area for the future functional food market. Therefore there are constant worries related to recovering and maintenance of healthy intestines, stocked with prebiotic nutrients that support the survival of beneficial health agents. This way, researchers and the segment of the food industry have encouraged the development of products with prebiotic properties, looking for the health promotion, treatment, and disease prevention, besides the strengthening of the competitive market. With consumer's increasing preference for products from natural origins, it is an important incentive for scientists and industrialists to seek prebiotics from natural sources. This growth has been enhanced by technological innovations, development of new products, and the increasing number of health-conscious consumers interested in products that improve life quality.

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00026-5
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2 Prebiotic dietary fiber sources

Various categories of prebiotic dietary fibers show different health benefits. FOSs, inulin, and GOSs have long been considered prebiotics. However, many other categories and compounds offer health benefits to consumers, although in varying ranges of efficacies. The following categories of prebiotic dietary fiber have sufficient evidence that they promote digestive health for consumers.

2.1 Beta-glucan

Beta-glucans are soluble compounds located in the endosperm cell walls of cereal grains, composed of linear D-glucopyranosyl units with a mixture of β-(1,3) and β-(1,4) glycosidic linkages and are also found in foods like mushrooms, algae, and other marine plants. Oat and barley are the two highest sources of beta-glucans today in the diet (Cloetens, Ulmius, Johansson-Persson, Akesson, & Onning, 2012). Because of their variation in terms of branching and length, beta-glucans can have a wide range of impacts on host GI health.

2.2 Inulin, oligofructose, and FOSs

Fructan compounds (β[2,1]-fructans) are found in a wide range of structures and foods. Inulin typically has a degree of polymerization (DP) between 3 and 60 fructan monomers. Oligofructose is made from the chemical degradation of these products with endoglycosidase enzymes, yielding a product with a DP of 2–20. FOSs are typically produced from the transfructosylation of sucrose and contain 2 and 4 β(2,1)-linked fructosyl units. All of these compounds exert strong bifidogenic effects, although the length of polymerization influences these effects (van deWiele, Boon, Possemiers, Jacobs, & Verstraete, 2007).

2.3 GOSs

GOSs are mixtures of oligosaccharides derived from the enzymatic glycosylation of primary lactose, primarily using β-galactosidases to catalyze various transgalactosylation reactions. Typical GOSs are composed of 2–10 molecules of galactose and 1 molecule of glucose, primarily synthesized from enzymatic activity. The purity, DP, type, and dosage of GOSs influence the prebiotic capacity in many clinical studies (Macfarlane, Steed, & Macfarlane, 2008).

2.4 Isomaltooligosaccharides

Isomaltooligosaccharides are glucose monomers linked by α(1,6)-glycosidic linkages made from the enzymatic treatment of cornstarch with α-amylase, pullulanase, and α-glucosidase, and its primary components are isomaltose, isomaltotriose, and panose. Depending on the final DP, effective doses range from 5 to 10 g/day for most individuals (Rastall, 2010). Doses as high as 30 g/day have also been shown to be tolerated in adults, with only mild GI side effects being noted (Wang et al., 2001).

2.5 Guar gum

Guar gum, in its intact state, is a gel-forming galactomannan made from the endosperm of the plant *Cyamopsis tetragonolobus* and is composed primarily of high molecular weight polysaccharides ([1,4]-linked β-D-mannopyranosyl units with [1,6]-linked α-D-galactopyranosyl side-chain residues) (Mudgil, Barak, & Khatkar, 2014). Guar is commonly used in dairy, bakery, cereal, and meat products.

2.6 Lactulose

Lactulose is a disaccharide composed of galactosyl β(1,4) fructose derived from the primary and secondary isomerization of lactose, neither digestible by mammalian enzymes nor hydrolyzed or absorbed in the small intestine. Although not found naturally in foods, limited clinical studies have consistently shown beneficial effects due to the fermentation of these compounds.

2.7 RS and maltodextrin

Resistant starch (RS) is a broad categorization of many classes of starches formed under a variety of conditions, but all escape digestion in the upper GI tract. Resistant maltodextrin is a low-viscosity, highly water-soluble dextrin that is

produced by treating cornstarch with numerous acid, enzymatic, and heating processes and used in a variety of applications. Some RSs are found naturally in foods, whereas others are purely synthetic (Sajilata, Singhal, & Kulkarni, 2006).

2.8 Xylooligosaccharides and arabinooligosaccharides

Xylooligosaccharides (XOSs) are composed of 2–10 xylose monomers linked with β(1,4) bonds with DP ≤20 (Voragen, 1998). XOSs are commonly found in dairy products, cereals, bars, sports drinks, and isotonic beverages. Doses of ≤12 g/day have been shown to be well tolerated in human intervention studies. Japan is responsible for nearly half of the production and consumption of XOSs worldwide, which have been approved as an ingredient for "Food for Specified Health Use" since 1991, and, according to regulation, are expected to have a specific effect on health (Vazquez, Alons, & Domınguez, 2000).

Arabinooligosaccharides (AOSs) are yet another class of oligosaccharides that hold the potential of being labeled as prebiotics. The exuberant source of AOS is arabinan polysaccharide—a branched pectic polysaccharide exhibiting linkage of 1,3-α-L-arabinofuranosyl and 1,5-α-L-arabinofuranosyl residues (Vogel, 1991). Arabinose occurs naturally in arabinans, arabinogalactans, or arabinoxylans present in plant cell wall components.

3 Prebiotic production from food industry wastes and agricultural by-products

A large number of by-products are abundantly produced annually worldwide from food processing and agricultural by-products. The disposal of these by-products confers negative impacts on the ecosystem while giving less opportunity for wealth creation. The agricultural and food processing by-products contain high nutritive values that can be used as the main source of functional or nutraceutical ingredients. In addition, a prebiotic differs from the classical dietary fibers, specifically soluble dietary fibers that selectively stimulate the growth and/or activity of beneficial bacteria, including probiotics, that is, *Bifidobacteria* and *Lactobacilli* species. These soluble dietary fibers have been shown to exert additional beneficial effects. They will improve gut barrier function in vitro and in vivo, which could be partially a consequence of their effect on the microflora composition. For example, pectic oligosaccharide (POS) and FOS formed from fruits and vegetables processing and the sugar cane industry, XOS from woods and corncobs, chitooligosaccharide from seafood processing, β-glucan from mushroom by-products, and soybean oligosaccharide from tofu and soy protein industry.

Currently, the top production of prebiotics at high volumes is inulin, followed by GOS. Prominent raw materials or substrates for the production of these prebiotics are roots of chicory and Jerusalem artichoke and lactose from the agriculture and dairy industries, respectively. However, due to high demand, the cost of these raw materials is exponentially increasing every year. Thus many manufacturers of prebiotics are shifting the utilization of their raw materials from other alternative sources at lower cost. Due to this situation, various by-products derived from food processing, including agricultural-based items, are of much interest in this solution.

Soybean whey, a by-product of tofu manufacturing, which is normally discarded, contains NDOs (nondigestible polysaccharides and oligosaccharides). Acidic fermentation of NDOs in the caecum leads to an increase in mineral absorption, especially that of calcium and magnesium. In view of its potential health-promoting properties, soybean whey may be used as a valuable ingredient in functional foods (Tenorio, Espinosa-Martos, & Prestamo, 2010). Prebiotic oligosaccharides extracted from soy sauce lees have growth-promoting effect on *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Yang, Hu, & Zhao, 2011a). Prebiotic oligosaccharides are obtained when water-extractable polysaccharides isolated from Bengal gram husk and wheat bran are subjected to driselase enzyme hydrolysis (Madhukumar & Muralikrishna, 2010).

The solid wastes are accumulated in malting industries, namely, barley husks, spent grains, and grain fragments; but when processed by hydrothermal techniques, the liquor contained XOSs. The refined oligosaccharides on fermentation generated succinate, lactate, formate, acetate, propionate, and butyrate, exhibiting prebiotic potential (Gullón, Gullón, Sanz, Alonso, & Parajó, 2011a). Wang, Liang, and Liang (2010) studied that mung bean may enhance the growth of *Lactobacillus paracasei*. Gullón, González-Muñoz, and Parajó (2011b) investigated the prebiotic potential of POS-rich refined product from apple pomace, processed by simultaneous saccharification and solid-state fermentation.

4 Development of prebiotic food products

Nowadays, one of the tendencies in the food segment is the healthiness and wellness, associated with the growth of the food industry for the more conscious consumer exigencies; an adequate feeding with healthy ingredients is indispensable to a better life. In recent times the production of potential prebiotics varies from diverse sources, ranging from agrowastes, dairy effluents, and industrial refuge to novel sources as seaweeds, cereal brand, and mushrooms. The list is vast and increasing.

Yacon (*Smallanthus sonchifolius*) root is a traditional food among the Andean population in South America. It has a myriad of health-promoting properties, including probiotic effects. Though European Union is skeptical about the safety of yacon, it has a well-documented and unambiguous history of safe use and it is free of toxic substances or antinutrients (Ojansivu, Ferreira, & Salminen Yacon, 2011). A very used ingredient, nowadays, in the development of new products is the yacon potato, tubercle from the Andes that has been featured because it is rich in FOS and inulin. Yacon can undergo different types of processing to obtain pulps, juices, and flours, and these are being added to other food. As adding yacon potato to breads, Rolim et al. (2011) verified a food rich in fiber, low glycemic index, and considerable prebiotic effect. Researchers also evaluated the effects of yacon potato flour addition in chocolate cakes, as an alternative in substituting sugar, and had positive results in terms of chemical composition, glycemic index and charge, and satisfactory prebiotic capacity (Padilha et al., 2010).

Huang, Cheng, Deng, Chou, and Jan (2012) investigated the effect of diosgenin, a steroid sapogenin compound from yam, on the growth of enteric lactic acid bacteria (LAB). Oral administration of diosgenin on the murine model restored the density of fecal LAB associated with food allergic reactions. The results indicate that the steroidal sapogenins may be a novel class of prebiotics. Lupin kernel fiber, a novel legume-derived food ingredient, is a prebiotic, modulating the colonic microbiota in humans, evident from the significantly higher levels of *Bifidobacterium* spp. and lower levels of the clostridia, namely, *Clostridium ramosum*, *Clostridium spiroforme*, and *Clostridium cocleatum* (Smith et al., 2006). Molan, Flanagan, Wei, and Moughan (2009a) reported the prebiotic effect of blueberry in terms of the increase in *Lactobacillus rhamnosus* and *Bifidobacterium breve*. It is hypothesized that the aqueous extract of blueberry could modify the bacterial profile by promoting the growth of beneficial bacteria and thereby improving gut health. Mandalari et al. (2007) studied the prebiotic effect of enzymatically derived POS-rich extract from bergamot peel and reported that *Bifidobacteria* and *Lactobacilli* responded positively to the addition of the extract. Prebiotic inulin-type fructans have been isolated from the roots of traditional Chinese medicine *Morinda officinalis* or Indian mulberry (Yang, Prasad, Xie, Lin, & Jiang, 2011b). The oligosaccharides of white- and red-flesh pitayas (dragon fruit) have been found capable of stimulating the growth of *Lactobacilli* and *Bifidobacteria* (Wichienchot, Jatupornpipat, & Rastall, 2010).The β-glucans extracted from *Pleurotus* sp. (pleuran) mushrooms have been used as food supplements due to their immunosuppressive activity and stimulation of probiotics. This exploitation of fruit body extracts extends the use of mushrooms *Pleurotus ostreatus* and *Pleurotus eryngii* for human health (Synytsya et al., 2009). Glycated pea proteins enhanced the growth of gut commensal bacteria, particularly *Lactobacilli* and *Bifidobacteria*, because the energy contained in glycated pea proteins, partially inaccessible for gastric enzymes, could be salvaged by gut microbiota. Such changes in the microbial composition may beneficially impact the intestinal environment and exert a health-promoting effect in humans (Dominika, Arjan, Karyn, & Henryk, 2011).

Molan, Lila, Mawson, and De (2009b) studied the effect of selenium-containing green tea (SGT) and China green tea (CGT) on the in vitro growth of *Lactobacilli* and *Bifidobacteria*. SGT showed significantly higher phenolic contents, higher reducing activity, higher DPPH free-radical scavenging activity, and higher ferrous-ion chelating activity than CGT. The addition of aqueous extracts of SGT to Mann–Rogosa–Sharpe (MRS) broth resulted in a significant increase in the numbers of *L. rhamnosus* and *B. breve*. The higher prebiotic activity of SGT over CGT may be related to the higher phenolic contents and minerals, notably selenium. The probiotic health potential of the polysaccharides from seaweeds is increasingly being studied in keeping with the increased consumption of whole seaweeds or the purified polysaccharides (Gupta & Abu-Ghannam, 2011). Ramnani et al. (2012) studied that low-molecular-weight polysaccharides derived from agar and alginate of seaweed *Gelidium* CC2253 showed a significant increase in bifidobacteria population. This seaweed easily fermented into short-chain fatty acid (SCFA) by gut bacteria and exhibits the potential to be used as a novel source of prebiotics. The sulfated heteropolysaccharide, ulvan, extracted from green algae *Ulva rigida* is resistant to both human digestive tract enzymes and degradation by colonic bacteria. So, it is expected that this polysaccharide can be deemed as a prebiotic and further hydrolyzed to functional oligosaccharides (O'Sullivan et al., 2010). Wang, Han, Hu, Li, and Yu (2006) studied that alginate oligosaccharide-rich diet stimulated the growth of *Bifidobacterium bifidum* ATCC 29521 and *Bifidobacterium longum* SMU 27001, decreasing the abundance of enterobacteriaceae and enterococci. Fructans from agave (*Agave tequilana* Weber var. azul) are designed to produce a mixture of functional prebiotic FOS and sweetening powder (Avila-Fernandez, Galicia-Lagunas, Rodriguez-Alegria, Olvera, & Lopez-Munguia, 2011). Neoagaro-oligosaccharides (NAOS), obtained from enzymatic hydrolysis of agarose, are found to be highly resistant to enzymes of the upper GI tract, which significantly stimulated the growth of bifidobacteria and lactobacilli. These results indicated that NAOS possessed great prebiotic effect that could be beneficial to the host (Hu et al., 2006). The water-soluble prebiotic compounds are found from Australian and New Zealand plants *Arthropodium cirratum* and *Cordyline australis* (Vidanarachchi, Iji, Mikkelsen, Sims, & Choct, 2009).

Cyanobacteria *Spirulina* sp. has huge nutritional value being a rich source of amino acids, high proteins, calcium, vitamins A, B2, B12, E, H, K, essential minerals, iron, fatty acids, and trace elements. The stimulatory effect of aqueous

suspensions of *Spirulina platensis* dry biomass has been reported on four LAB in milk. The addition of dry *S. platensis* to milk (6 mg/mL) stimulated the growth of *Lactococcus lactis*, to a remarkable 27% (de Caire, Parada, Zaccaro, & de Cano, 2000). Nowadays, when the dairy industry is supplementing milk with minerals, vitamins, and antioxidants, it would be of interest to consider the possibility of adding *Spirulina* biomass to fermented milk to induce a faster production of LAB in the dairy product as well as in the gut. Xylanase from fungi *Hypocrea jecorina* cleaved the arabinoxylan fraction of the cereal resulting in arabinoxylan oligosaccharides (AXOS) content of 2.1%. Rye or wheat bran fortification in the AXOS-rich dough further enhanced bread quality. The prebiotic effect of the fermented cashew apple juice, containing oligosaccharides, is evaluated through the *Lactobacillus johnsonii* B-2178 growth (Vergara, Honorato, Maia, & Rodrigues, 2010). The addition of lactulose in skim milk increased the probiotic counts (particularly *Bifidobacterium lactis*), the acidification rate, and amount of lactic acid acidity, concurrently reducing the fermentation time (Oliveira, Florence, Perego, Oliveira, & Converti, 2010). Functional *petit-suisse* cheese, made with the combination of prebiotics (inulin, oligofructose, and honey) and probiotics (*Lactobacillus acidophilus* La-5 and *B. lactis* BL04), was found to be more health friendly, as fast fermentation and high lactic acid production along with increased growth rates of *Bifidobacteria* and *Lactobacilli* were observed (Cardarelli, Saad, Gibson, & Vulevic, 2007).

Ice creams supplemented with *Lactobacillus casei* and 2.5% inulin showed good nutritional and sensory properties (Criscio et al., 2010). Roble, Brunton, Gormley, Ross, and Butler (2010) developed potentially synbiotic fresh-cut apple wedges by applying probiotic bacteria (*L. rhamnosus* GG) and prebiotics oligofructose and inulin. Fructan analysis showed that all prebiotics remained stable over the 14-day storage period and an intake of 100 g of apple supplied 2–3 g of prebiotics. Different dehydrated prebiotic fibers, namely, oat bran, β-glucan, and green banana flour, provided substratum for adherence and trehalose acted as a cell protectant prolonging the viability of *L. casei*. In the sensory evaluation, the prebiotic oat bran added to a dairy fruit beverage has been well accepted by consumers (Guergoletto, Magnani, Martin, Andrade, & Garcia, 2010). Apple purees enriched with two commercial FOS prebiotics [Beneo GR® (inulin) and HSI®] showed stability for 30-day storage at 4°C (Keenan, Brunton, Butler, Wouters, & Gormley, 2011). However, ample quantities of prebiotics were required to deliver prebiotic effect, and high hydrostatic pressure posed the risk of certain prebiotics hydrolysis. Buckwheat diet showed an increase in aerobic mesophilic and LAB content compared with control. *Lactobacillus plantarum*, *Bifidobacterium* spp., and *B. lactis* were found in buckwheat diet, confirming its candidature as a prebiotic product (Prestamo, Pedrazuela, Penas, Lasuncion, & Arroyo, 2003). Buckwheat diet also decreased the level of cholesterols in serum by enhancing their removal through feces, improved diabetes by inhibiting the absorption of sugars, and enhanced the growth of beneficial intestinal bacteria. D-Chiro-inositol, found in buckwheat, contributed to the improvement of insulin resistance by potentiating the action of insulin (Takahama, Tanaka, & Hirota, 2011). Lyophilized inulin syrup made from Jerusalem artichoke tuber was used as a prebiotic ingredient in the small-scale manufacture of wafer crackers. Sensory analysis revealed a significant influence of product formulation on appearance, flavor, and texture of the crackers (Hempel, Jacob, & Rohm, 2006). The impact of the incorporation of prebiotic AXOS on the quality of a sugar-snap cookie type was investigated. Replacing up to 30% of the initial sucrose level by AXOS resulted in cookies with comparable size, but of darker color than the control cookies. Pareyt, Goovaerts, Broekaert, and Delcour (2011) proposed the possible role of AXOS as a sucrose substitute, which has practical implications from a health point of view, reasonable consumption of which may exert beneficial biological effects. Dietary fibers exhibiting high viscoelasticity impart breads with better sensory perception, lower digestible starch, and higher RS contents, lowering down the in vitro expected glycemic index (Angioloni & Collar, 2011). Damen et al. (2012) investigated the in situ production of prebiotic AXOS during bread making. Peach-flavored yogurts containing the prebiotic did not significantly influence the flavor and so did not much impact consumer acceptance (Gonzalez, Adhikari, & Sancho-Madriz, 2011). Allgeyer, Miller, and Lee (2010) studied the popularity of dairy products fortified with prebiotics and probiotics and motivated with the consumer desire for good-flavored healthy foods. Sensory profile of drinkable yogurts made with prebiotics, namely, soluble corn fiber, polydextrose, and chicory inulin, was assessed. Polydextrose treatment seemed to be an acceptable vehicle to deliver the probiotic health effects at the end of the 30-day storage period. Mitsou, Panopoulou, Turunen, Spiliotis, and Kyriacou (2010) evaluated the in vivo probiotic potential of barley β-glucan and concluded that it induced a strong bifidogenic effect. It is reported that the daily intake of a cake containing barley β-glucan is well tolerated and demonstrated significant bifidogenic properties in older healthy volunteers.

Human milk oligosaccharides (HMOs) are increasingly being recognized as a vital prebiotics for infant well-being. However, its complexity in terms of structural and functional diversity restricts its inclusion in dietary ingredients. Now, enzymatic production of some of its components is being carried out with moderate success. Monomers *N*-acetylglucosamine and fucose are identified as an important factor. Separation and concentration of HMO-like oligosaccharides from bovine milk are also being carried out (Barile & Rastall, 2013). Baumgartner, Seitz, Sprenger, and Albermann (2013) engineered an *Escherichia coli* capable of producing lacto-*N*-tetraose (a component of HMO) by chromosomal integration of the genes lgtA and wbgO, encoding β-1,3-*N*-acetylglucosaminyltransferase and β-1,3-galactosyltransferase. Prebiotics

TABLE 26.1 Functional properties of prebiotics in foods.

Applications	Functional properties
Yoghurts and desserts	Sugar replacement, texture and mouthfeel, fiber, and prebiotics
Beverages and drinks	Sugar replacement, mouthfeel, foam stabilization, and prebiotics
Breads and fillings	Fat or sugar replacement, texture, fiber, and prebiotics
Meat products	Fat replacement, texture, stability, and fiber
Dietetic products	Fat or sugar replacement, fiber, and prebiotics
Cake and biscuits	Sugar replacement, moisture retention, fiber, and prebiotics
Chocolate	Sugar replacement, heat resistance, and fiber
Sugar confectionary	Sugar replacement, fiber, and prebiotics
Soups and sauces	Sugar replacement, and prebiotics
Baby food	Texture, body and mouthfeel, fiber, stability, and prebiotics

have also being used in child formulation (Millani, Konstantyner, & Taddei, 2009) in a way to answer children's questions about not have access to mother's milk or partially received it. Consumption of these products evidence improvements in allergy cases, crying and colic increase, and improvement in the prevention of constipation, among others. However, the food industry must be cautious to incorporate prebiotics to child food, considering that studies are still necessary for this area. Macdonald and Monteleone (2005) reported that a diet designed for infants having phenylketonuria with prebiotic GOS/FOS oligosaccharide blend of 0.8 g/100 mL maintained serum phenylalanine levels, enhanced the levels of bifidobacteria, and lowered stool pH.

Prebiotics can be incorporated into many foodstuffs as agents to improve or maintain a balanced intestinal microflora to enhance health and well-being. In food formulations, they can also significantly improve organoleptic characteristics, upgrading both taste and mouthfeel. Food applications of prebiotics are illustrated in Table 26.1.

The prebiotic effect of a new product, models may be used. Samples can be submitted to the process of fermentation with the anaerobic system and the addition of an inoculum prepared from the infant's feces. The liquid is used for metabolic bacterial count homo- and heterofermentative, as well as providing analysis of SCFAs. To count the bacteria, we can use the differential medium agar also generating system anaerobic, and analysis of SCFA can be performed by gas chromatography (Vanderzant & Splittsloesser, 2001). It is also possible to measure the effect of a prebiotic potential by evaluating the resistance of microorganisms to acid, which can be done with MRS broth with accompanying the pH. Another method to evaluate microorganisms is the resistance to bile using lyophilized bovine bile for monitoring the cell viability at different times (Ronka et al., 2003).

Promising research with prebiotics can be produced by sugar solution through transfructosylated reaction (Aachary & Prapulla, 2009). The solid wastes accumulated in industries when sucrose-saturated solutions can synthesize FOSs (Gullón et al., 2011b). Rodrigues, Rocha-Santos, Gomes, Goodfellow, and Freitas (2012) investigated the effect of FOS and inulin (50:50) on the free fatty acid profile of cheese and reported an increase in the conjugated linoleic acid content (known to have several health benefits). However, the generation from all sources is not economically viable. The discovery of cost-effective approaches for the conversion and recovery of prebiotics is required. The prevalent current technology for the production of prebiotic oligosaccharides is acid, enzymatic, and microwave hydrolysis of polysaccharides sourced from plants or microbes or synthesis by transglycosylation reactions employing microbial enzymes (Avila-Fernandez et al., 2011). Cloning and expressing genes coding for enzymes catalyzing the conversion of polysaccharides into oligosaccharides has also received some success, namely, glucosidase gene from *Thermoanaerobacter ethanolicus* expressed in *E. coli*, and β-hexosyltransferase gene from *Sporobolomyces singularis* expressed in yeast *Pichia pastoris* (Wang, Yi, Yu, Dong, & Qiao, 2009). Enzyme immobilization on various matrices has also resulted in a higher yield of oligosaccharides (Quang et al., 2011). Roasting, baking, and boiling increased the RS content of processed cereals and its products (Vaidya & Sheth, 2010).

5 Prebiotics safety

Prebiotics are assumed to lack life-threatening or severe side effects. Intestinal enzymes cannot break down oligosaccharides and polysaccharides. They are transported to the colon to be fermented by the gut microbiota. Therefore the side effects of prebiotics are mostly the result of their osmotic functions. In this regard, osmotic diarrhea, bloating, cramping,

and flatulence could be experienced in prebiotic recipients. The prebiotics chain length is an influential parameter for the development of their side effects. Interestingly, prebiotics with shorter chain length may have more side effects. The possible explanation for this phenomenon is that shorter inulin molecules are metabolized primarily in the proximal colon and are more rapidly fermented, whereas longer chain ones are fermented later and slower in the distal colon. Besides chain length the prebiotic dose can affect its safety profile. For example, low (2.5–10 g/day) and high (40–50 g/day) doses of prebiotics can cause flatulence and osmotic diarrhea, respectively. Noting that a daily dose of 2.5–10 g prebiotics is required to exert their beneficial functions on human health. This means that prebiotics within their therapeutic doses can cause mild-to-moderate side effects. Most products of prebiotics in the market have doses of 1.5–5 g per portion (Svensson & Håkansson, 2014).

The safety of inulin-type fructans and galactose and their sources as food or food ingredients is not debated on the basis of their long-term use. However, the novel emerging prebiotics still need further consideration of their safety in humans. The ecological consideration has significance for the safety of prebiotics (Hammes & Hertel, 2002). Generally, prebiotics are assumed safe. However, alterations in the intestinal microflora could result in adverse effects, depending on what bacterial populations are stimulated. Moreover, the shift of the metabolism toward enhanced butyrate formation may have some disadvantageous consequences, for example, by supporting the growth of undesired clostridia (Wang, 2009). A recent study was carried out to assess the tolerance and safety of a formula containing an innovative mixture of oligosaccharides in early infancy (Piemontese et al., 2011). Formula-fed infants were randomly fed with a regular formula containing a mixture of neutral oligosaccharides and pectin-derived acidic oligosaccharides (prebiotic formula group). The authors demonstrated the tolerability and the long-term safety of such potential prebiotic formula in a large cohort of healthy infants.

6 Food applications of prebiotics

Prebiotics are polysaccharides that are capable of surviving acidic and enzymatic digestion in the small intestine and can be fermented by probiotics in the large intestine to produce SCFAs, vitamins, and other compounds, which can normalize bowel movement, increase immunity against diseases, prevent cancer, improve mineral absorption, and lower cholesterol. Health benefits from prebiotics may be obtained by incorporating them into products such as nutraceuticals and functional foods. The followings are some examples of the products that have taken advantage of prebiotics:

1. Functional beverages containing inulin and oligofructose to improve the efficiency of the digestive system, strengthen the bone, and increase immunity against diseases.
2. Breakfast cereals with an increased amount of dietary fibers through the addition of prebiotics increase the efficiency of the digestive system and enhance the growth of probiotics.
3. Infant food with an addition of prebiotics improves the efficiency of the digestive system and increases the immunity against diseases. Addition of inulin, oligofructose, and GOSs, individually or in combination, to infant food, has been shown to increase the numbers of *Bifidobacteria* and *Lactobacilli* in the infant's digestive system from 31% to 59% of total gut microflora during their first 6 weeks, which is similar to breastfeeding.
4. Dairy products, for example, fermented dairy beverages and yoghurt containing probiotics, which become known as synbiotics when prebiotics are added. Among other things, they help one to improve Ca absorption.
5. Nutraceuticals, where prebiotics are made into tablets or capsules; some also contain probiotics. They are taken daily to increase the amount of pro- and/or prebiotics in the GI system.
6. Other products, for example, weight management products, where sugar is replaced by prebiotics; some have a similar sweetness than sugar.

Special care must be taken in using prebiotics as ingredients in food products. The compatibility of these ingredients with the products in terms of physical (particle size, solubility, and viscosity), sensory (color, taste, and flavor), and nutritional (health benefits, potential hazards, dosage, and stability) properties must be well understood. If properly used, they can be an additional tool to combat ailments and to improve the health and well-being of consumers.

Prebiotics show both important technological characteristics and interesting nutritional properties. In food formulations, they can significantly improve sensory characteristics, upgrading both taste and mouthfeel. Aside from having the function of fiber and prebiotic in itself, prebiotics have been successfully used in foods on the basis of their following properties: sugar and fat replacement, texture improvement, body and mouthfeel, moisture retention, heat resistance, foam stabilization, stability to food processing conditions, and others (Wang, 2009). In relation to the nutritional properties, for prebiotics to serve as functional food ingredients, they must be chemically stable to food processing treatments such as heat, low pH, and Maillard reaction conditions. Stability to processing conditions is essential if prebiotics are to confer beneficial effects for the host. That is, a prebiotic would no longer provide selective stimulation of beneficial microorganisms if it was

TABLE 26.2 Marketed prebiotics.

Food product	Company	Prebiotics	Dosage form
Nutren Fiber®	Nestle	Inulin	Powder
Miracle Fiber	The Vitamin Shoppe, North Bergen, NJ	Inulin	Powder
Kal NutraFlora FOS	Nutraceutical Corp, Park City, UT	Short-chain fructooligosaccharides	Powder
Solaray NutraFlora FOS	Nutraceutical Corp Short-chain	Fructooligosaccharides	Capsules
Fiber Choice	GlaxoSmithKline, Philadelphia, PA	Inulin	Chewable tablet/drops
Smart Fiber Stixx	Intelligent Nutrition LLC, New Orleans, LA	Oligofructose and inulin	Powder
Balance, Prebiotic Fiber	pHion	Inulin fiber; psyllium seed husk (*Plantago ovata*)	Powder
Organic, Inulin	Now Foods	Organic inulin (FOS)	Powder
Frutafit® HD	Sensus	Inulin FOS	Powder
Frutalose® L85	Sensus	Oligofructose	Syrup
Vivinal GOS	Friesland	Galactooligosaccharides	Syrup

degraded to its component mono- and disaccharides or chemically altered so that it was unavailable for bacterial metabolism. In contrast, processing may cause a chemical alteration of the prebiotic that may result in increased selective enrichment of beneficial colonic bacteria (Huebner, Wehling, Parkhurst, & Hutkins, 2008).

When incorporating prebiotics in food, one must take into account the particular characteristics of each prebiotic. Among these characteristics the fermentation rate, the type of affected microbiota, the recommended dose for a prebiotic effect, and the maximum dose that does not induce adverse effects based on scientific studies and by law must be considered. One must also take into account its stability and its technological features, which are directly related to the physicochemical, rheological, and sensory attributes of a particular product where the prebiotic would be added.

The growth-promoting effect of prebiotics has been extensively explored by administering them as such in a dosage form. Table 26.2 lists only a few examples of the never-ending list of available prebiotic products. However, a recent trend is to coencapsulate them with one or more probiotics to achieve a synbiotic preparation. By doing so, encapsulated prebiotics not only promote the growth of existing gut microflora but also act as growth supporter for entrapped probiotic on storage (Patel, Singh, Panaich, & Cardozo, 2014). The concept of synbiotic has been extensively explored for carbohydrate type prebiotics like inulin and FOSs (Slavin, 2013). However, these prebiotics apart from encouraging the growth of probiotic bacteria may equally favor nonprobiotic pathogenic bacteria residing in the gut (Bello, Walter, Hertel, & Hammes, 2001) and the latter trait is a concern. In order to selectively stimulate probiotic growth, exploring newer noncarbohydrate selective prebiotic sources need to be explored. Recently, some polyphenols have shown promising results (Lopez de Lacey, Perez-Santin, Lopez-Caballero, & Montero, 2014).

Generally, the Asian continent has been the leading consumer of fermented functional foods with Japan being the leading market in the region (Nakakuki, 2002). The worldwide market of prebiotics was estimated to reach US$ 155.41 billion after 2010, with a yearly growth potential of 10% (Otieno & Ahring, 2012).

7 Conclusion

Prebiotics can enhance the growth and activities of probiotics, and gut microflora, which are beneficial to the health and well-being of humans and animals. They may be incorporated as nutritional ingredients into various functional foods or presented as nutraceuticals in the form of tablets or capsules, sometimes with probiotic cultures, for the benefit of consumer's health. More recently, researchers have begun to unravel at least some of the functional and nutritional interactions between members of the colonic ecosystem and this is providing us with new outcomes to target in prebiotic interventions. This coincides with a new focus on the products of bacterial metabolism as important indicators of prebiotic action. When creating functional products with added prebiotics, it is important to determine the most suitable prebiotic for the particular food product being developed. The particular characteristics of the prebiotics should be taken into account, including their nutritional and technological properties such as stability to processing conditions, minimum and maximum concentrations that lead to the desirable effects, and the adverse effects at the concentration range underuse. The health benefits associated

with positive technological applications are the main reasons for the widespread use of prebiotics in today's human diet. The introduction of functional compounds like prebiotics in the diet seems to be an attractive alternative. Owing to its wide range of preventive and therapeutic possibilities, prebiotics research is certainly catching the momentum.

References

Aachary, A. A., & Prapulla, S. G. (2009). Value addition to spent osmotic sugar (SOS) by enzymatic conversion to fructooligosaccharides (FOS), a low calorie prebiotic. *Innovative Food Science and Emerging Technologies*, *10*, 284–288.

Allgeyer, L. C., Miller, M. J., & Lee, S. Y. (2010). Sensory and microbiological quality of yogurt drinks with prebiotics and probiotics. *Dairy Science*, *93*, 4471–4479.

Angioloni, A., & Collar, C. (2011). Physicochemical and nutritional properties of reduced-caloric density high-fibre breads. *LWT—Food Science and Technology*, *44*, 747–758.

Avila-Fernandez, A., Galicia-Lagunas, N., Rodriguez-Alegria, M. E., Olvera, C., & Lopez-Munguia, A. (2011). Production of functional oligosaccharides through limited acid hydrolysis of agave fructans. *Food Chemistry*, *129*, 380–386.

Barile, D., & Rastall, R. A. (2013). Human milk and related oligosaccharides as prebiotics. *Current Opinion in Biotechnology*, *24*, 214–219.

Baumgartner, F., Seitz, L., Sprenger, G. A., & Albermann, C. (2013). Construction of *Escherichia coli* strains with chromosomally integrated expression cassettes for the synthesis of 2′-fucosyllactose. *Microbial Cell Factories*, *12*, 40.

Bello, F. D., Walter, J., Hertel, C., & Hammes, W. P. (2001). *In vitro* study of prebiotic properties of levan-type exopolysaccharides from *Lactobacilli* and non-digestible carbohydrates using denaturing gradient gel electrophoresis. *Systematic and Applied Microbiology*, *24*, 232–237.

Burgain, J., Gaiani, C., Linder, M., & Scher, J. (2011). Encapsulation of probiotic living cells: From laboratory scale to industrial applications. *Journal of Food Engineering*, *104*(4), 467–483.

Cardarelli, H. R., Saad, S. M. I., Gibson, G. R., & Vulevic, J. (2007). Functional petit-suisse cheese: Measure of the prebiotic effect. *Anaerobe*, *13*, 200–207.

Cloetens, L., Ulmius, M., Johansson-Persson, A., Akesson, B., & Onning, G. (2012). Role of dietary beta-glucans in the prevention of the metabolic syndrome. *Nutrition Reviews*, *70*(8), 444–458.

Coman, M. M., Cecchini, C., Verdenelli, M. C., Silvi, S., Orpianesi, C., & Cresci, A. (2012). Functional foods as carriers for SYNBIO®, a probiotic bacteria combination. *International Journal of Food Microbiology*, *157*(3), 346–352.

Criscio, T. D., Fratianni, A., Mignogna, R., Cinquanta, L., Coppola, R., Sorrentino, E., et al. (2010). Production of functional probiotic, prebiotic, and synbiotic ice creams. *Journal of Dairy Science*, *93*, 4555–4564.

Damen, A., Pollet, A., Dornez, E., Broekaert, W. F., Van Haesendonck, I., Trogh, I., et al. (2012). Xylanase mediated in situ production of arabinoxylan oligosaccharides with prebiotic potential in whole meal breads enriched with arabinoxylan rich materials. *Food Chemistry*, *131*, 111–118.

de Caire, G. Z., Parada, J. L., Zaccaro, M. C., & de Cano, M. M. S. (2000). Effect of *Spirulina platensis* biomass on the growth of lactic acid bacteria in milk. *World Journal of Microbiology and Biotechnology*, *16*, 563–565.

Dominika, S., Arjan, N., Karyn, R., & Henryk, K. (2011). The study on the impact of glycated pea proteins on human intestinal bacteria. *International Journal of Food Microbiology*, *145*, 267–272.

Gibson, G. R., Ottaway, P. B., & Rastall, R. A. (2000). In *Prebiotics: New developments in functional foods* (pp. 1–327). Oxford, UK: Chandos Publishing Ltd.

Gibson, G. R. (1995). Dietary modulation of the human colonic microbiota – Introducing the concept of prebiotics. *Journal of Nutrition*, *125*(6), 1401–1412.

Guergoletto, K. B., Magnani, M., Martin, J. S., Andrade, C. G. T. D., & Garcia, S. (2010). Survival of *Lactobacillus casei* (LC-1) adhered to prebiotic vegetal fibres. *Innovative Food Science and Emerging Technologies*, *11*, 415–421.

Gullón, B., Gullón, P., Sanz, Y., Alonso, J. L., & Parajó, J. C. (2011 ba). Prebiotic potential of a refined product containing pectic oligosaccharides. *LWT—Food Science and Technology*, *44*, 1687–1696.

Gullón, P., González-Muñoz, M. J., & Parajó, J. C. (2011 ab). Manufacture and prebiotic potential of oligosaccharides derived from industrial solid wastes. *Bioresource Technology*, *102*, 6112–6119.

Gonzalez, N. J., Adhikari, K., & Sancho-Madriz, M. F. (2011). Sensory characteristics of peach-flavored yogurt drinks containing prebiotics and synbiotics. *LWT—Food Science and Technology*, *44*, 158–163.

Gupta, S., & Abu-Ghannam, N. (2011). Bioactive potential and possible health effects of edible brown seaweeds. *Trends in Food Science and Technology*, *22*, 315–326.

Hammes, W. P., & Hertel, C. (2002). Research approaches for pre- and probiotics: Challenges and outlook. *Food Research International*, *35*, 165–170.

Hempel, S., Jacob, A., & Rohm, H. (2006). Influence of inulin modification and flour type on the sensory quality of prebiotic wafer crackers. *European Food Research and Technology*, *224*, 335–341.

Huang, C. H., Cheng, J. Y., Deng, M. C., Chou, C. H., & Jan, T. R. (2012). Prebiotic effect of diosgenin, an immunoactive steroidal sapogenin of the Chinese yam. *Food Chemistry*, *132*, 428–432.

Huebner, J., Wehling, R. L., Parkhurst, A., & Hutkins, R. W. (2008). Effect of processing conditions on the prebiotics activity of commercial prebiotics. *International Dairy Journal*, *18*, 287–293.

Hu, B., Gong, Q., Wang, Y., Ma, Y., Li, J., & Yu, W. (2006). Prebiotic effects of neoagaro-oligosaccharides prepared by enzymatic hydrolysis of agarose. *Anaerobe*, *12*, 260–266.

Keenan, D. F., Brunton, N., Butler, F., Wouters, R., & Gormley, R. (2011). Evaluation of thermal and high hydrostatic pressure processed apple purees enriched with prebiotic inclusions. *Innovative Food Science and Emerging Technologies*, *12*, 261–268.

Lopez de Lacey, A. M., Perez-Santin, E., Lopez-Caballero, M. E., & Montero, P. (2014). Survival and metabolic activity of probiotic bacteria in green tea. *LWT—Food Science and Technology*, *55*, 314–322.

Macdonald, T. T., & Monteleone, G. (2005). Immunity, inflammation, and allergy in the gut. *Science*, *307*(5715), 1920–1925.

Macfarlane, G., Steed, H., & Macfarlane, S. (2008). Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. *Journal of Applied Microbiology*, *104*(2), 305–344.

Madhukumar, M. S., & Muralikrishna, G. (2010). Structural characterization and determination of prebiotic activity of purified xylooligosaccharides obtained from Bengal gram husk (*Cicer arietinum* L.) and wheat bran (*Triticum aestivum*). *Food Chemistry*, *118*, 215–222.

Mandalari, G., Palop, C. N., Tuohy, K., Gibson, G. R., Bennett, R. N., Waldron, K. W., et al. (2007). In vitro evaluation of the prebiotic activity of a pectic oligosaccharide-rich extract enzymatically derived from bergamot peel. *Applied Microbiology and Biotechnology*, *73*, 1173–1179.

Mitsou, E. K., Panopoulou, N., Turunen, K., Spiliotis, V., & Kyriacou, A. (2010). Prebiotic potential of barley derived β-glucan at low intake levels: a randomized, double-blinded placebo-controlled clinical study. *Food Research International*, *43*, 1086–1092.

Millani, E., Konstantyner, T., & Taddei, J. A. A. C. (2009). Effects of prebiotics (oligosaccharides) on child's health. *Revista Paulista de Pediatria*, *27*(4), 436–446.

Molan, A. L., Flanagan, J., Wei, W., & Moughan, P. J. (2009 ba). Selenium containing green tea has higher antioxidant and prebiotic activities than regular green tea. *Food Chemistry*, *114*, 829–835.

Molan, A. L., Lila, M. A., Mawson, J., & De, S. (2009 ab). In vitro and in vivo evaluation of the prebiotic activity of water-soluble blueberry extracts. *World Journal of Microbiology and Biotechnology*, *25*, 1243–1249.

Mudgil, D., Barak, S., & Khatkar, B. S. (2014). Guar gum: Processing, properties and food applications—A review. *Journal of Food Science and Technology*, *51*(3), 409–418.

Nakakuki, T. (2002). Present status and future of functional oligosaccharide development in Japan. *Pure and Applied Chemistry*, *74*, 1245–1251.

Ojansivu, I., Ferreira, C. L., & Salminen Yacon, S. (2011). A new source of prebiotic oligosaccharides with a history of safe use. *Trends in Food Science and Technology*, *22*, 40–46.

Oliveira, R. P. D. S., Florence, A. C. R., Perego, P., Oliveira, M. N. D., & Converti, A. (2010). Use of lactulose as prebiotic and its influence on the growth, acidification profile and viable counts of different probiotics in fermented skim milk. *International Journal of Medical Microbiology*, *145*, 22–27.

O'Sullivan, L., Murphy, B., McLoughlin, P., Duggan, P., Lawlor, P. G., Hughes, H., et al. (2010). Prebiotics from marine macroalgae for human and animal health applications. *Marine Drugs*, *8*, 2038–2064.

Otieno, D. O., & Ahring, B. K. (2012). The potential for oligosaccharide production from the hemicellulose fraction of biomasses through pretreatment processes: Xylooligosaccharides (XOS), arabinooligosaccharides (AOS), and mannooligosaccharides (MOS). *Carbohydrate Research*, *360*, 84–92.

Padilha, V. M., Rolim, P. M., Salgado, S. M., Livera, A. S., Andrade, S. A. C., & Guerra, N. B. (2010). Sensorial profile of chocolate cakes formulated with yacon flour (*Smallanthus sonchifolius*). *Food Science and Technology (Campinas)*, *30*(3), 735–740.

Panitantum, V. (2004). The story of probiotics, prebiotics & synbiotics. In *A seminar presentation at Kasetsart University, Bangkok, under the auspices of BIOTEC* (pp. 157–161).

Pareyt, B., Goovaerts, M., Broekaert, W. F., & Delcour, J. A. (2011). Arabinoxylan oligosaccharides (AXOS) as a potential sucrose replacer in sugar-snap cookies. *LWT—Food Science and Technology*, *44*, 725–728.

Patel, P. J., Singh, S. K., Panaich, S., & Cardozo, L. (2014). The aging gut and the role of prebiotics, probiotics, and synbiotics: A review. *Journal of Clinical Gerontology and Geriatrics*, *5*, 3–6.

Piemontese, P., Giannì, M. L., Braegger, C. P., Chirico, G., Grüber, C., Riedler, J., et al. (2011). Tolerance and safety evaluation in a large cohort of healthy infants fed an innovative prebiotic formula: A randomized controlled trial. *PLoS One*, *6*(11), e28010.

Prestamo, G., Pedrazuela, A., Penas, E., Lasuncion, M. A., & Arroyo, G. (2003). Role of buckwheat diet on rats as prebiotic and healthy food. *Nutrition Research*, *23*, 803–814.

Quang, D., Nguyen, Q. D., Judit, M., Rezessy-Szabo, J. M., Czukor, B., & Hoschke, A. (2011). Continuous production of oligofructose syrup from Jerusalem artichoke juice by immobilized endo-inulinase. *Process Biochemistry*, *46*, 298–303.

Ramnani, P., Chitarrari, R., Tuohy, K., Grant, J., Hotchkiss, S., Philp, K., et al. (2012). In vitro fermentation and prebiotic potential of novel low molecular weight polysaccharides derived from agar and alginate seaweeds. *Anaerobe*, *18*(1), 1–6.

Rastall, R. A. (2010). Functional oligosaccharides: Application and manufacture. *Annual Review of Food Science and Technology*, *1*, 305–339.

Roble, C., Brunton, N., Gormley, R. T., Ross, P. R., & Butler, F. (2010). Development of potentially synbiotic fresh-cut apple slices. *Journal of Functional Foods*, *2*, 245–254.

Rodrigues, D., Rocha-Santos, T. A. P., Gomes, A. M., Goodfellow, B. J., & Freitas, A. C. (2012). Lipolysis in probiotic and symbiotic cheese: The influence of probiotic bacteria, prebiotic compounds and ripening time on free fatty acid profiles. *Food Chemistry*, *131*(4), 1414–1421.

Rolim, P. M., Salgado, S. M., Padilha, V. M., Livera, A. V. S., Andrade, S. A. C., & Guerra, N. B. (2011). Glycemic profile and prebiotic potential "in vitro" of bread with yacon (*Smallanthus sonchifolius*) flour. *Food Science and Technology (Campinas)*, *31*(2), 467–474.

Ronka, E., Malinen, E., Saarela, M., Rinta-Koski, M., Aarnikunnas, J., & Palva, A. (2003). Probiotic and milk technological properties of *Lactobacillus brevis*. *International Journal of Food Microbiology*, *83*(1), 63–74.

Sajilata, M. G., Singhal, R. S., & Kulkarni, P. R. (2006). Resistant starch? A review. *Comprehensive Reviews in Food Science and Food Safety*, *5*(1), 1–17.

Slavin, J. (2013). Fibre and prebiotics: Mechanisms and health benefits. *Nutrients*, *5*, 1417–1435.

Smith, S. C., Choy, R., Johnson, S. K., Hall, R. S., Wileboer-Veloo, A. C., & Welling, G. W. (2006). Lupin kernel fiber consumption modifies fecal microbiota in healthy men as determined by rRNA gene florescent in situ hybridization. *European Journal of Nutrition*, *45*, 335–341.

Synytsya, A., Mıckova, K., Synytsya, A., Jablonsky, I., Spevacek, J., Erban, V., et al. (2009). Glucans from fruit bodies of cultivated mushrooms *Pleurotus ostreatus* and *Pleurotus eryngii*: Structure and potential prebiotic activity. *Carbohydrate Polymers*, *76*, 548–556.

Svensson, U., & Håkansson, J. (2014). *Safety of food and beverages: Safety of probiotics and prebiotics*. New York, NY, USA: Elsevier.

Takahama, U., Tanaka, M., & Hirota, S. (2011). Flour and breads and their fortification in health and disease prevention. In V. R. Preedy, R. R. Watson, & V. B. Patel (Eds.), *Buckwheat flour and bread* (pp. 141–151). London: Academic Press.

Tenorio, M. D., Espinosa-Martos, I., & Prestamo, G. (2010). Soyabean whey enhance mineral balance and caecal fermentation in rats. *European Journal of Nutrition*, *49*, 155–163.

Vaidya, R. H., & Sheth, M. K. (2010). Processing and storage of Indian cereal and cereal products alters its resistant starch content. *Journal of Food Science and Technology*, *48*, 622–627.

Vanderzant, C., & Splittsloesser, D. F. (2001). In *Compendium of methods for the microbiological examination of foods* (4th ed.). Washington, DC: American Public Health Association.

van deWiele, T., Boon, N., Possemiers, S., Jacobs, H., & Verstraete, W. (2007). Inulin-type fructans of longer degree of polymerization exert more pronounced *in vitro* prebiotic effects. *Journal of Applied Microbiology*, *102*(2), 452–460.

Vazquez, M., Alons, J., & Domınguez, H. (2000). Xylooligosaccharides: Manufacture and applications. *Trends in Food Science & Technology*, *11*(11), 387–393.

Vergara, C. M. A. C., Honorato, T. L., Maia, G. A., & Rodrigues, S. (2010). Prebiotic effect of fermented cashew apple (*Anacardium occidentale* L) juice. *LWT—Food Science and Technology*, *43*, 141–145.

Vernazza, C. L., Rabiu, B. A., & Gibson, G. R. (2006). Human colonic microbiology and the role of dietary intervention: Introduction to prebiotic. In G. R. Gibson, & R. A. Rastall (Eds.), *Prebiotics: Development & application* (pp. 1–28). West Sussex, England: John Wiley & Sons, Ltd.

Vidanarachchi, J. K., Iji, P. A., Mikkelsen, L. L., Sims, I., & Choct, M. (2009). Isolation and characterization of water-soluble prebiotic compounds from Australian and New Zealand plants. *Carbohydrate Polymers*, *77*, 670–676.

Vogel, M. (1991). Alternative utilisation of sugar beet pulp. *Zuckerindustrie*, *116*, 266–270.

Voragen, A. (1998). Technological aspects of functional food-related carbohydrates. *Trends in Food Science & Technology*, *9*(8–9), 328–335.

Wang, Y. (2009). Prebiotics: Present and future in food science and technology. *Food Research International*, *42*(1), 8–12.

Wang, Y., Han, F., Hu, B., Li, J., & Yu, W. (2006). In vivo prebiotic properties of alginate oligosaccharides prepared through enzymatic hydrolysis of alginate. *Nutrition Research*, *26*, 597–603.

Wang, H. F., Lim, P. S., Kao, M. D., Chan, E. C., Lin, L. C., & Wang, N. P. (2001). Use of isomalto-oligosaccharide in the treatment of lipid profiles and constipation in hemodialysis patients. *Journal of Renal Nutrition*, *11*(2), 73–79.

Wang, S. L., Liang, Y. C., & Liang, T. W. (2010). Purification and characterization of a novel alkali-stable amylase from *Chryseobacterium taeanense* TKU001 and application in antioxidant and prebiotic. *Process Biochemistry*, *46*, 745–750.

Wang, A. N., Yi, X. W., Yu, H. F., Dong, B., & Qiao, S. Y. (2009). Free radical scavenging activity of *Lactobacillus fermentum* in vitro and its antioxidative effect on growing-finishing pigs. *Journal of Applied Microbiology*, *107*, 1140–1148.

Wichienchot, S., Jatupornpipat, M., & Rastall, R. A. (2010). Oligosaccharides of pitaya (dragon fruit) flesh and their prebiotic properties. *Food Chemistry*, *120*, 850–857.

Yang, Z., Hu, J., & Zhao, M. (2011 ba). Isolation and quantitative determination of inulin-type oligosaccharides in roots of *Morinda officinalis*. *Carbohydrate Polymers*, *83*, 1997–2004.

Yang, B., Prasad, K. N., Xie, H., Lin, S., & Jiang, Y. (2011 ab). Structural characteristics of oligosaccharides from soy sauce lees and their potential prebiotic effect on lactic acid bacteria. *Food Chemistry*, *126*, 590–594.

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Chapter 27

Therapeutic Potential of Different Probiotic Foods

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1 Introduction

Microbes are ubiquitous throughout the globe. Their presence makes the life sometimes easier and sometimes harder. Probiotics are one such microbe that makes the human life healthier. Probiotics are achieved through the process of fermentation. Fermentation is as old as the humanity itself. By the action of enzymes, organic substances are transformed into simpler compounds that are more easily digestible. The word fermentation means "to boil" which was derived from *Latin.* Fermentation has its root from as early as Neolithic times with the production of beer, wine, leavened bread, and cheese, subsequently followed by fermented foods, yogurt, pickles, etc.

This chapter is a critical analysis of the therapeutic potential of different traditionally unique and widely popular probiotic foods used by varied populations. The scientific studies carried out far and wide have proved that these different probiotic foods have aided in the cure of many ailments and pathologies, improving the health of consumers considerably. Collectively known as functional foods, these probiotics, prebiotics, and synbiotics, as they are otherwise referred to, are extremely nutritious and beneficial to the host. They are normally consumed as part of the diet but have proven physiological benefits like improving the gastrointestinal flora, thereby promoting not only gut health but also improving overall well-being as well. Beyond their nutritive function, they have also been shown to carry potential bioactive compounds that have the ability to reduce the risk of chronic diseases. This chapter highlights the various probiotic food and beverages like curd, yoghurt, kefir, kimchi, kombucha, miso, tempeh, sauerkraut, idli, and appam and their significant components for the improvement of human health and the aspect of their being used as therapeutic agents in disease prevention and cure.

1.1 Probiotics

The etymology of probiotics means "for life" in the Greek language, but the definition of the term has been widely expanding each day and evolving over time. The term "probiotics" was coined by Lilly and Stanwell in 1965. Initially probiotics referred to a substance or a group of substances produced by certain microbial strains that enhanced the growth of other beneficial microbes and helped one to balance the intestinal flora to produce positive effects. But the present updated definition given by the Food and Agricultural Organization (FAO) of the UN says that probiotics are "live microorganisms that, when administered in adequate quantity can confer a health benefit in the host" (FAO guidelines, 2002). This definition has also been accepted by the International Scientific Association of Probiotics and Prebiotics in 2103. In relation to food, this definition says that the benefits are attained when consumed in adequate amount as part of the food. According to their usage or application, there are subcategories of probiotics, namely, probiotic drugs, probiotic foods, probiotics as animal feed, and genetically engineered probiotics.

1.2 Prebiotics

Prebiotics according to the FAO/World Health Organization (WHO) are defined as "nonviable food component that confer health benefits on the host associated with modulation of the microbiota." They are also widely explored as potential nutrients and supplements with beneficial effects like probiotics. The different sources of prebiotics are as follows: breast

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00027-7
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milk, soybeans, inulin from diverse sources (Jerusalem artichoke, chicory roots), raw oat, wheat bran, barley bran, yacon roots, and nondigestible carbohydrates (nondigestible oligosaccharides). From prebiotics, only bifidogenic, nondigestible oligosaccharides, especially inulin, and its hydrolysis products, such as oligofructose, and (*trans*) galactooligosaccharides (GOS), are suitably qualified as prebiotic foods. Prebiotics are not only obtained naturally from sources like vegetables, fruits, and grains consumed in our daily life but are also artificially synthesized such as: lactulose, GOS, and fructooligosaccharides. An ideal prebiotic should be resistant to the actions of acids in the stomach, bile salts, and other hydrolyzing enzymes in the intestine; not be absorbed in the upper gastrointestinal tract; and be easily fermentable by the beneficial intestinal microflora.

1.3 Synbiotics

The advances in microbial research has given rise to another new category known as "synbiotics" that are a synergistic combination of probiotics and prebiotics that provide the survival and the implantation of the live microorganism dietary supplements in the gut. The symbiotic associations of both the probiotic and prebiotic act together in the living system and improve the human health. From the medical point of view, it is a "synbiotic product" that promotes the survival and implantation of live microbial dietary supplements into the gastrointestinal tract and stimulates the growth and metabolism of health promoting bacteria.

2 Criteria for the selection of probiotic food

For the living organisms to be termed as probiotics, the given criteria should be fulfilled:

1. It must be separated from the same species of its expected host.
2. It must have a positive impact on its host.
3. It should not produce any symptoms of disease.
4. It should have the capacity to survive in the extreme acidic conditions of the gastrointestinal tract and should easily pass through it.
5. During storage the microscopic organisms must have the capacity to endure extended lengths of time.

3 Different types of probiotic food

Fermentation of the food is carried by the bacteria which is naturally present in it. When lactic fermentation acts upon the starch and the sugar present in the food, it produces probiotic bacteria along with fatty acid, enzyme, and vitamin and also converts the food into more digestible form Gitanjali and Manda (2016).

3.1 Classification of fermented food is based on different substrates

Milk-based: Yogurt, cheese, and kefir
Cereal- and legume-based: Idli, dosa, and appam
Legume-based: Tempeh and miso
Vegetable- and fruit-based: Kombucha, pickles, kimchi, and sauerkraut

3.2 Milk-based: Yogurt, cheese, and kefir

3.2.1 Yogurt

Yogurt is one of the best known of the foods that contain probiotics. Yogurt is defined by the *Codex Alimentarius* of 1992 as a coagulated milk product that results from the fermentation of lactic acid in milk by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Bourlioux & Pochart, 1988). Yogurt is also a nutrient-dense probiotic food, with unique properties that enhance the bioavailability of some of the nutrients potentially enhancing health (El-Abbadi, Dao, & Meydani, 2014). Yoghurt is a product of the lactic acid fermentation of milk by addition of a starter culture containing *S. thermophilus* and *Lactobacillus delbrueckii* ssp. *Bulgaricus*. In some countries, less traditional microorganisms, such as *Lactobacillus helveticus* and *L. delbrueckii* ssp. *lactis*, are sometimes mixed with the starter culture (Tamime, 2002). As starter cultures for yogurt production, lactic acid bacteria (LAB) species display symbiotic relations during their growth in milk medium (Chandan & Shahani, 1993). Thus a carefully selected mixture of LAB species is used to complement each other and to achieve a remarkable efficiency in acid production. In many modern societies, fermented dairy products make up a

substantial proportion of the total daily food consumption. Furthermore, it has long been believed that consuming yogurt and other fermented milk products provides various health benefits (Metchnikoff, 1901).

Yogurt can be commercially produced with substantial variety in composition, flavors, and additives. These include whole-milk, low-fat, or nonfat forms; plain or flavored; inclusion of fruit; addition of natural or artificial sweeteners; and occasional supplementation with vitamin D (Codex, 2001; FAO, 2003). Numerous studies suggested beneficial therapeutic effects of LAB on gut health. However, results have been inconsistent, which may be due to differences in the strains of LAB, routes of administration, and investigational procedures used in these studies (Adolfsson, Meydani, & Russell, 2004b).

3.2.1.1 Nutritional value of yogurt

The nutrient composition of yogurt is based on the nutrient composition of the milk from which it is derived, which is affected by many factors, such as genetic and individual mammalian differences, feed, stage of lactation, age, and environmental factors such as the season of the year. Other variables that play a role during processing of milk, including temperature, duration of heat exposure, exposure to light, and storage conditions, also affect the nutritional value of the final product. In addition, the changes in milk constituents that occur during lactic acid fermentation influence the nutritional and physiologic value of the finished yogurt product. The final nutritional composition of yogurt is also affected by the species and strains of bacteria used in the fermentation, the source and type of milk solids that may be added before fermentation, and the temperature and duration of the fermentation process.

Dairy products have generally been considered an excellent source of high-quality protein, calcium, potassium, phosphorus, magnesium, zinc, and the B vitamins riboflavin, niacin, vitamin B-6, and vitamin B-12 (Buttriss, 1997). Dairy products and foods prepared with the use of dairy ingredients are an exclusive source of the disaccharide lactose in human diets (Chandan & Shahani, 1993). One example of a significant bacteria-induced change that occurs during the fermentation process is the hydrolysis of 20–30% of the disaccharide lactose to its absorbable monosaccharide components, glucose and galactose (Bourlioux & Pochart, 1988). In addition, a portion of the glucose is converted to lactic acid. Depending on other ingredients added, this hydrolysis results in lower lactose concentrations in yogurt than in milk, which in part explains why yogurt is tolerated better than milk by persons with lactose maldigestion (Rosado, Solomons, & Allen, 1992; Vesa, Marteau, & Korpela, 2000; Kolars, Levitt, Aouji, & Savaiano, 1984). In another example, bacteria present in yogurt, such as *L. bulgaricus* and *S. thermophilus*, expressed functional lactase, the enzyme that breaks down lactose (Goodenough & Kleyn, 1976). This expression may also contribute to better tolerance of lactose in yogurt than of lactose in milk by persons with lactose maldigestion (Kolars et al., 1984).

Yogurt is abundant in calcium, zinc, B vitamins, and probiotics; it is a good source of protein; and it may be supplemented with vitamin D and additional probiotics associated with positive health outcomes.

Vitamins: Yogurt is also a good source of B vitamins: a 100-g serving of plain low-fat yogurt contains riboflavin, niacin, vitamin B-6, and vitamin B-12 at amounts of 0.21, 0.11, 0.05, and 0.56 mg, respectively (USDA, Agricultural Research Service, 2013).

Protein: Yogurt is an excellent source of essential amino acids of high biological quality and generally contains higher protein content than milk. Furthermore, the proteolytic activity of bacterial cultures in yogurt enables some predigestion of milk proteins, resulting in greater amounts of free amino acids that allow for better protein digestibility (Adolfsson et al., 2004). Protein from yogurt is more easily digested than is protein from milk, as bacterial predigestion of milk proteins in yogurt may occur (Shahani & Chandan, 1979; Rasic & Kurmann, 1978). This argument is supported by evidence of a higher content of free amino acids, especially proline and glycine, in yogurt than in milk (Loones, 1989). Similarly, niacin and riboflavin levels in yogurt are increased with fermentation (Deeth & Tamime, 1981; Alm, 1982).

Lipids: Yogurt has been shown to have a higher concentration of conjugated linoleic acid (CLA), a long-chain biohydrogenated derivative of linoleic acid, than does the milk from which the yogurt was processed (Shantha, Ram, O'Leary, Hicks, & Decker, 1995). A fermented dairy product from India, referred to as dahi, has also been shown to have higher CLA content (Fig. 29.1). CLA was reported to have immunostimulatory and anticarcinogenic properties (Whigham, Cook, & Atkinson, 2000).

Minerals: In addition to being a good source of protein, yogurt is an excellent source of calcium and phosphorus (Block & Abrams, 1993). In the rat model, calcium retention was greater with the consumption of a diet in which lactose made up half the total carbohydrates ingested than with the consumption of the control diet (Kaup, Shahani, Amer, & Peo, 1987; Schaafsma, Dekker, & de Ward, 1988) and, investigating the effect of dairy products on mineral absorption by using rat models, reported that lactose enhances the absorption of calcium, magnesium, and zinc. The acidic pH of yogurt ionizes calcium and thus facilitates intestinal calcium uptake (Bronner & Pansu, 1999).

3.2.1.2 Therapeutic role of yogurt

Few studies have investigated the effect of yogurt-derived calcium on bone mineralization in animals. Kaup et al. reported that yogurt-fed rats showed greater bone mineralization than did rats fed a diet containing calcium carbonate. These studies may suggest that the bioavailability of calcium in yogurt is greater and yogurt may increase bone mineralization in human; however, currently there are no published results (Kaup et al., 1987; Pointillart, Cayron, & Gueguen, 1986; Bernet, Brassart, Neeser, & Servin, 1994). In a group of elderly patients with atrophic gastritis and hypochlorhydria, *Lactobacillus gasseri* survived passage through the gastrointestinal tract, but *S. thermophilus* and *L. bulgaricus* were not recovered (Pedrosa et al., 1995).

The ability of LAB to decrease the gastrointestinal invasion of pathogenic bacteria has also been described (Bernet et al., 1994; Bernet, Brassart, Neeser, & Servin, 1993). Orally administered *Lactobacillus acidophilus* and *Lactobacillus casei* and the feeding of yogurt increased both IgA production and the number of cells secreting IgA in the small intestine of mice in a dose-dependent manner (Perdigon, Alvarez, Rachid, Aguero, & Gobbato, 1995). Similarly, a report by Puri, Rattan, Bijlani, Mahapatra, and Nath (1996) indicated that *Salmonella typhimurium*–induced serum IgA concentrations were significantly higher in yogurt-fed mice. This report suggests that the IgA secreted by the challenged intestinal B cells enters the circulation and increases the concentrations of IgA in the serum. Thus the IgA-enhancing effect of yogurt intake may have both an effect on the gut and a systemic effect. The same study also showed that intestinal lymphocytes from yogurt-fed mice had a higher mitogen-induced proliferative response after a challenge with *S. typhimurium*. In a study using human subjects, Link-Amster, Rochat, Saudan, Mignot, and Aeschlimann (1994) showed that the specific anti-IgA titer to *S. typhimurium* was four times greater in subjects fed fermented milk containing *L. acidophilus* than in control subjects fed diets without fermented milk. Total sIgA concentrations also increased in subjects fed fermented milk. Several reports indicated that consumption of yogurt or intake of LAB by themselves modulates the production of several cytokines, such as IL-1_, IL-6, IL-10, IL-12, IFN-_, and TNF-_ Moreover, the production of IFN-_ in an in vitro culture system using human lymphocytes was reported to be greater with cultures in the presence of LAB (*L. bulgaricus* and *S. thermophilus*) than with those without LAB. Yogurt containing live *L. bulgaricus* and *S. thermophilus* was also reported to augment IFN-_ production by purified T cells from young adults after 4 months feeding (Miettinen, Vuopio-Varkila, & Varkila, 1996; Solis-Pereyra, Aattouri, & Lemonnier, 1997; Halpern, Vruwink, Van de Water, Keen, & Gershwin, 1991; Borruel, Carol, & Casellas, 2002; De Simone, Salvadori, Negri, Ferrazzi, Baldinelli, & Vesely, 1986).

Earlier evidence has shown that higher consumption of dairy products, which are rich in calcium, may protect adults against bone loss (McCabe et al., 2004). Yogurt may be an important source of protein and calcium in the elderly that could potentially lead to enhancement in bone and muscle health (Corthier, 2004). Yogurt extract has also been found to protect against demineralization and even enhance remineralization of tooth enamel in vitro (Ferrazzano et al., 2008).

3.2.1.3 Yogurt and the gastrointestinal tract

Diarrheal diseases diarrhea is a common problem among children worldwide and has been reported to contribute substantially to pediatric physician visits and hospitalizations in the United States (Glass, Lew, Gangarosa, LeBaron, & Ho, 1991). Since the early 20th century, it has been hypothesized that live bacterial cultures, such as those used for the fermentation of dairy products, may offer benefits in preventing and treating diarrhea (Metchnikoff, 1901).

Colon cancer: Fermented dairy products or the bacteria used for milk fermentation were shown to have an effect on colon cancer and certain other tumors in murine models of carcinogenesis (Reddy & Rivenson, 1993; Ayebo, Shahani, & Dam, 1981; Shackelford, Rao, Chawan, & Pulusani, 1983; Wollowski, Ji, Bakalinsky, Neudecker, & Pool-Zobel, 1999). Feeding of yogurt or *Lactobacillus* reduced fecal enzymes that convert procarcinogens to carcinogens, such as azoreductase and nitroreductase (Pedrosa et al., 1995).

Inflammatory bowel disease (IBD): Studies have shown an improvement in symptoms of IBD, pouchitis, and ulcerative colitis with the consumption of certain strains of lactobacilli (Parvez, Malik, Ah Kang, & Kim, 2006).

Obesity: Epidemiologic studies indicate that dairy products, and in particular yogurt, may have the potential to reduce the risk of obesity (Mozaffarian, Hao, Rimm, Willett, & Hu, 2011). In a study to assess the cross-sectional association of yogurt with diet quality and metabolic profile in w6500 participants in the Framingham Offspring Study, Wang, Livingston, Fox, Meigs, and Jacques (2013) found that yogurt consumers, on average, exhibited significantly improved metabolic variables, including lower BMI and blood pressure, reduced concentrations of triglycerides, fasting glucose, and insulin and greater high-density lipoprotein (HDL) cholesterol compared with nonconsumers, after adjustment for demographic and lifestyle factors that included diet quality. Evidence from randomized controlled trials (RCTs) in overweight and obese individuals further indicates that dietary calcium, particularly when sourced from dairy, can promote weight and fat loss (Zemel et al., 2005).

Inflammatory and cardio metabolic diseases: The only one to do so found that yogurt-enriched diets resulted in reduced C-reactive protein and increased adiponectin concentrations compared with controls (Zemel et al., 2005; Labonte, Couture, Richard, Desroches, & Lamarche, 2013). Results showed that yogurt consumers have significantly reduced prevalence of nutrient deficiencies for riboflavin, vitamin B-12, calcium, magnesium, and zinc (Wang et al., 2013).

Lactose intolerance: Research has shown that lactose in yoghurt is tolerated better by individuals with hypolactasia than an equivalent quantity in milk (Mckinley, 2005).

Helicobacter pylori: Several in vitro and animal studies have shown reduced viability of *H. pylori* and less adhesion of the bacteria to human intestinal mucosal cells after treatment with various *Lactobacillus* strains (Aiba, Suzuki, Kabir, Takagi, & Koga, 1998). In series of in vitro assays, Midolo, Lambert, Hull, Luo, and Grayson (1995) showed that the growth of *H. pylori* was inhibited by lactic acid in a pH-independent manner. They also found that six strains of *L. acidophilus* and *L. casei* inhibited the growth of *H. pylori*, whereas *Bifidobacterium bifidum* and *L. bulgaricus* did not. The role(s) of probiotics bacteria in dairy fermentations is to assist in (1) the preservation of the milk by the generation of lactic acid and possibly antimicrobial compounds; (2) the production of flavor compounds (e.g., acetaldehyde in yoghurt and cheese) and other metabolites (e.g., extracellular polysaccharides) that will provide a product with the organoleptic properties desired by the consumer; (3) to improve the nutritional value of food, as in, for example, the release of free amino acids or the synthesis of vitamins; and (4) the provision of special therapeutic or prophylactic properties as cancer and control of serum cholesterol levels. Metchnikoff (1901) was perhaps the first researcher to propose that fermented dairy products have beneficial properties. Fermentation of food with LAB increases folic acid in yogurt, bifidus milk, and kefir (Parvez et al., 2006).

3.2.2 Cheese

3.2.2.1 Cottage cheese and soft cheese

In 2006 the term "probiotic" was used by the dairy industries for more than 600 products they launched (Sveje, 2007). Fermented milk and yogurts were the best examples of diary-based probiotic food that ought to be consumed within a few week of manufacturing (Nagpal et al., 2007). Cheese is the generic name for a group of fermented milk-based food products produced throughout the world in a great diversity of flavors, textures, and forms (Fox, Guinee, Cogan, & McSweeney, 2000).

Cheese is one of the most versatile food products available nowadays, appealing to many palates and suitable for all age groups. Its versatility offers opportunities for many marketing strategies (Wilkinson, Meehan, Stanton, & Cowan, 2001), as a probiotic food carrier. The true origin of cheese is a mystery. Sheep and cattle were domesticated in Egypt and Mesopotamia so many years ago and the Old Testament refer to it as "Cheese of kine." The basic principle for cheese making remain the same and production process can be referred to as a dehydration process, since there is the draining away of the whey. The conversion of milk to cheese involves several distinct but interrelated processes, including coagulation, acidification, synthesis (separation of the whey from the cheese), dehydration, molding, pressing, salting, and ripening. Cheese is the concentrated form of milk containing most of calcium, protein, fat, and vitamins, which are needed by man for the development of strong bones and for healthy living. Cheese is a concentrated source of many of the nutrients in milk. The enzymes and bacteria involved in ripening of each specific kind of cheese may also alter the nutrient content of the end product (Ogilvy, 1977; Lomholt & Qvist, 1999). Consumption of cheese has grown in the past decade in most countries, unrelated to the socioeconomic level of the country. Cheese from cow's milk represents 95%–96% of the total cheese production (IDF, 2007).

3.2.2.2 Overview of cheese making

The manufacture of cheese is a form of milk preservation, as milk is highly perishable. All cheeses, whether rennet or acid set, can be classified as soft, semisoft (semihard), hard, or very hard, depending on their moisture content (Farke, 2004). The production of cheese requires the coagulation of milk, in most cases through the action of chymosin on the k-casein steric stabilizing layer of the casein micelle. Cheese production is a process of the dehydration of milk in combination with preservative procedure, such as culturing, acidification, salting, packaging, and refrigeration (Everett & Auty, 2008). The rennet-induced milk coagulum is cut and homogenized to expel moisture in a process called syneresis (Grundelius, Lodaite, Östergren, Paulsson, & Dejmek, 2000). Curd is later drained, salted, and packaged into fresh cheese. Many cheeses need an additional time to achieve their own sensory features, particularly flavor and aroma. To achieve this purpose, they are maintained in a special room, with controlled environmental conditions for a determined time: this process is called ripening and the final product is called ripened cheese (Everett & Auty, 2008). Cheese is a probiotic food carrier (Stanton et al., 2003). In fact, cheese provides a valuable alternative to fermented milks and yogurts as a food vehicle for probiotic delivery. It creates a buffer against the high acidic environment in the gastrointestinal tract by creating a more favorable environment

for probiotic survival throughout the gastric transit, due to higher pH. Moreover, the dense matrix and relatively high fat content of cheese may offer additional protection to probiotic bacteria in the stomach, which was confirmed by Sharp et al. (da Cruz, Buriti, de Souza, Faria, & Saad, 2009; Sharp, McMahon, & Broadbent, 2008). It was reported that the ingestion of probiotic cheese in animals and humans was as effective as yogurt to deliver viable cells of *Enterococcus faecium* during its ripening period (Gardiner et al., 1999).

Soft cheeses fall into two categories; ripened soft cheese such as brie and fresh cheese and unripened cheese such as cottage cheese. Soft cheeses are made from heat-treated milk which is fermented by the use of specific bacteria. Artificial rennet is sometimes used to produce a firmer texture. Fresh cheeses are ready for use as soon as the manufacturing process is complete but ripened cheeses are allowed to mature in temperature- and humidity-controlled rooms for up to 1 month (John and Omojate, 2013).

3.2.2.3 Nutritional value of cheese

Nutritious rich food cheese is made mostly from the milk of cows, buffaloes, sheeps, or goats, and it is an important food component in the healthy diet of America, Asia, and Europe (Ash & Wilbey, 2010). Cheese serves as one of the good sources for essential nutrients, such as proteins, lipids, minerals, and vitamins. It can enhance health benefits by the production of certain peptides and free amino acids which has various bioactive properties, such as antimicrobial, anticarcinogenic, and antithrombotic. Certain biologically active lipids, such as conjugated linoleic acid (CLA) which is a conjugated fatty acid and milk fat globule membrane (MFGM), present in cheeses are also found to be health beneficial (Kwak & Palanivel, 2011). A large variety of cheeses have been produced in the world to meet the requirements of taste and health need of individuals. But their consumption varies with individual, race, ethnicity of people, and type of milk in cheese preparation. In recent years, cheese found to be health beneficial due to the presence of health-promoting factors, such as essential amino acids and free fatty acids.

Numerous factors that include type of milk, lactation period, ripening process, and type of starter used can vary the nutritional components of the cheese. The fully ripened cheese contains highly water-soluble components than its source, milk (Kosikowski & Mistry, 1997). It also contains several peptides that have various bioactive roles in humans. These peptides can be generated not only during the ripening process of cheese but also during the digestion process in human tract. Bioactive peptides (BPs) generated during the ripening as well as during digestion are found to be health beneficial, such as angiotensin-converting enzyme (ACE)-inhibitory activity (Smacchi & Gobbetti, 1998).

The potential and the nutrient significant of cheese are of wide range due to the presence of potentially significant amount of both saturated and unsaturated fat. Certain fats in cheese are found to be health beneficial, such as CLA, which shows various biological activities that include anticancer, antithrombotic, antidiabetic, and antiatherosclerosis. The average content of CLA in cheese is found to be 0.5–1.7 g/100 g of free fatty acids. The various beneficial roles of CLA include body fat reduction, inhibition of tumor, antiatherosclerosis, and antiobesity (Park & Pariza, 2007). Caseins in cheese are of nutritionally rich due to the high supply of essential amino acids, phosphate, and calcium. They also act as a good source of energy in the human diet. The nutrition value of cheese protein was slightly lower than the milk protein due to the loss of sulfur containing protein in whey during cheese making process. Thus cheeses provide a good source of peptides and amino acids from casein and thereby prevent the dismantling of vital proteins in our body.

Lactoferrin is a multifunctional glycoprotein existed in the cheese at the range of 672 μg/g (soft cheese)–1218 μg/g (semihard cheese) (Dupont et al., 2006). These proteins have various physiological roles that include host defense against variety of microorganisms, iron homeostasis, anticancer, antiviral, and antiinflammatory activity (Elbarbary, Abdou, Park, Nakamura, & Mohamed, 2010). Cheese flavor and texture are mostly contributed by the proteolysis of cheese protein that leads to the generation of various biologically and nutritionally rich peptides and amino acids. The proteolysis actions are mostly catalyzed by the enzymes, such as proteases and peptidases (Kunji, Mierau, Hagting, Poolman, & Konings, 1996). Cheese can produce BPs not only during the ripening process but also during the digestion of cheese in the gastrointestinal tract on in vitro conditions (Parrot, Degraeve, Curia, & Martial-Gros, 2003). These BPs have a positive impact on the health of human, and their activity is based on their amino acid composition and their ability to reach the target site. BPs from cheese show hypotensive and/or ACE1 inhibition (Gomez-Ruiz, Ramos, & Recio, 2002), antihypertensive activity, antithrombotic activity, anticarcinogenic activity, opioid activities, stimulation of immunity, and so on (Butikofer, Meyer, Sieber, & Wechsler, 2007). Small amount of *trans*–fatty acids are found in cheese and are found to be health beneficial (Kuhlsen et al., 2005).

Mineral concentration in cheese varies with the type of cheese, manufacturing procedure, coagulation method, and the amount of salt added in cheese (Lucey & Fox, 1993). Calcium is an important macromineral for our body and it is present abundance in cheese (Kruif & Holt, 2006). Cheese also acts as good source of phosphorous that exist in various forms such as soluble phosphate exists in the serum phase and bound phosphate exists in the paracasein part of cheese curd (Upretia

& Metzge, 2007). The vitamin content is variable between types and variety of cheese. It also depends on the milk vitamins, microbial cultures, and also the conditions in which cheese is matured (Oste, Jagerstad, & Andersson, 1997). Milk vitamins, such as vitamin A and D, are retained mostly in the cheese curd together with other fat-soluble vitamin (Favier & Dorsainvil, 1987).

3.2.2.4 Therapeutic role of cheese

Probiotic fresh cheese (Argentinean fresh cheese) containing *L. acidophilus* A9, *B. bifidum* A12, and *Lactobacillus paracasei* A13 demonstrated immunomodulating capacity in mice, providing increased phagocytic activity in the small intestine of peritoneal macrophages, after 2, 5, and 7 days of its ingestion. Additionally, a significant increase in the number of IgAþ producing cells in the large intestine after 5 days of administration was reported (Medici, Vinderola, & Perdigon, 2004).

A study was conducted on the effect of probiotic Edam cheese containing *Lactobacillus rhamnosus* LC705 and *L. rhamnosus* GG ATCC53103 (LGG) on the risk of dental caries. The authors concluded that eating probiotic cheese could reduce the risk of dental caries in general, although no significant statistical difference was observed in salivary microbial populations (Ahola et al., 2002). An investigation was made on the effect of consumption of probiotic cheese on the oral candidosis for 92 elderly people, where the cheese containing a mixture of probiotic cultures (*L. rhamnosus* GG, *L. rhamnosus* LC705, *Propionibacterium freudenreichii* spp.) was ingested. Microbiological analyses of oral yeasts were performed in saliva samples and found that the probiotic treatment reduced the risk of high yeast populations by 75%. The authors hypothesized that probiotic bacteria have somehow affected the composition of saliva, the concentrations of mucins and salivary immunoglobulins. They suggested that probiotic cheese could be used as a prophylactic approach for decreasing the risk of hyposalivation and the feeling of dry mouth, and that it could be considered beneficial to oral health in general (Hatakka et al., 2007).

Some of the cheese LAB species show antigenotoxic and antimutagenic properties reducing the risk of cancer (Hsieh & Chou, 2006; Massi, Vitali, Federici, Matteuzzi, & Brigidi, 2004). Among the LAB species, *Lactobacilli casei* isolated from traditional Italian ewe cheeses show microbial antigenotoxic–antimutagenic properties (Caldini, Trotta, Corsetti, & Cenci, 2008; Corsetti et al., 2008). Thus cheese also serves as an alternative functional with the implementation of certain beneficial bacteria, and further research also should be focused to enhance the value of these functional cheese based on the disease-specific functions.

3.2.3 Kefir

Kefir is a popular fermented milk drink consumed all around the world. It is believed to have originated from the Northern Caucasus mountains in the former Soviet Union and was very popular in Hungary and Poland for many years, even though it is only now becoming popular in other regions of the world. It is also well known in Sweden, Norway, Finland, and Germany, as well as in Greece, Austria, Brazil, and Israel. Nowadays, it is gaining popularity in the United States and Japan. The name "kefir" is derived from the Turkish word "keyif" meaning "good feeling" that is used to describe the feeling a person gets after drinking it. It improves the overall health of a person.

Yoghurt is a fermentation of milk with bacteria, whereas kefir uses a combination of bacteria and yeast. Kefir is got from the fermentation of milk with kefir grains that look like small lumps of coral. Commonly cow's milk is used in kefir preparation but other types of milk like sheep, goat, coconut, rice, and soy milk can also be used. The milk is fermented using kefir grains directly or using mother culture from the grains. The fermented product contains various components like lactic acid, acetic acid, CO_2, ethyl alcohol, and aromatic compounds resulting in a fizzy acid like taste and flavor (Otles & Cagindi, 2003). In short, it is an acid-fermented drink with a mild alcoholic sour taste. Another form of kefir is called "water kefir" that is not made of milk but uses water, sugar, and water kefir grains are also prevalent today. But this is different from the traditional kefir and there is very limited evidence of its use (Dimidi, Rose Cox, Rossi, & Whelan, 2019).

Other kefir-related products include freeze–dried kefir, made from concentrated milk (360 g/kg total solids) and fermented using traditional kefir grains; buttermilk kefir, another traditional product made from skimmed milk; and cultured milk kefir made from baker's yeast along with cream or yogurt starter culture. In many countries, kefir-like products with varying properties are produced, which look like kefir but are not similar to traditional kefir. These include Omaere (in South-West Africa), Rob or Roba (in some Arab countries), KjaKlder MjoKlk (in Norway), Kellermilch (in Germany), Tarag (in Mongolia), and Osobyi, a modified kefir produced in Russia (Otles & Cagindi, 2003).

3.2.3.1 Preparation of kefir

Kefir is prepared traditionally using kefir grains or mother cultures of kefir grains. Kefir grains are prepared in a goat-hide bag filled with pasteurized milk and inoculated with sheep intestinal flora, followed by culture of the surface layer in milk. Gradually a polysaccharide layer appears on the surface of the hide. The layer is removed from the hides and propagated in

pasteurized milk. Kefir grains appear as small cauliflower like florets, like gelatinous yellow or white particles, of 3–20 mm diameter (Libudzisz & Piatkiewicz, 1990). It is made up of 13% protein by dry weight and 24% polysaccharide, the principal one being "kefiran," a water-soluble substance.

The traditional method of making kefir is by adding the kefir grains to raw boiled milk and cooled to 20°C–25°C and inoculated with 2%–10% (generally 5%) kefir grain. After a period of fermentation, 18–24 hours at 20°C–25°C, the grains are separated from the milk by filtering with a sieve and can be dried at room temperature and kept at cold temperature for being used in the next inoculation. Kefir is stored at 4°C for a time then is ready for consumption. Industrial kefir uses the same principle basically except that heat treatment is done at 90°C–95°C for 5–10 minutes to homogenize the milk, followed by fermentation. It is distributed into bottles. Then it is allowed to mature at 12°C–14°C for 24 hours and then stored at 4°C.

3.2.3.2 Nutritional value of kefir

The composition of kefir depends on the source and fat content of the milk used. The chemical composition of kefir is basically lactic acid, carbon dioxide, and alcohol formed during fermentation. Diacetyl and acetaldehyde are produced by *Streptococcus lactis* subsp. diacetylactis and *Leuconostoc* species. The pH of kefir is 4.2–4.6 (Otles & Cagindi, 2003).

The beneficial microbes found in kefir include bacteria and yeast in a symbiotic association with each other. The common microbes in kefir include *Lactobacillus brevis*, *L. paracasei*, *L. helveticus*, *Lactobacillus kefiranofaciens*, *Lactobacillus plantarum*, *Lactobacillus kefiri*, *Lactococcus lactis*, *S. thermophilus*, *Acetobacter lovaniensis*, *Acetobacter orientalis*, *Saccharomyces cerevisiae*, *Saccharomyces unisporus*, *Candida kefyr*, *Kluyveromyces marxianus*, and *Leuconostoc mesenteroides* (Dimidi et al., 2019). It contains a complex mixture of bacteria like various species of *Lactobacilli*, *Lactococci*, *Acetobacteria*, and *Leuconostocs* and lactose-fermenting and nonlactose-fermenting yeasts. Homofermentative *Lactobacillus* species, including *L. kefiranofaciens* and *L. kefiri* (Toba et al., 1987; Yokoi et al., 1991), are present in kefir grains. The recommended value of the FAO and the WHO is that kefir grains should contain a minimum 107 colony-forming units (CFU)/g microorganisms and the final product should contain at least 104 CFU/g of yeast (Dimidi et al., 2019).

Many vitamins and minerals are richly present in kefir which have several health benefits. Kefir contains vitamin B_1, B_{12}, calcium, amino acids, folic acid, and vitamin K. It is a good source of biotin, a B vitamin that aids the body's assimilation of other B vitamins, such as folic acid, pantothenic acid, and B12.

3.2.3.3 Therapeutic potential of kefir

The abundance of vitamins in kefir helps the regulation of the kidneys, liver, and nervous system, relieves skin disorders, boosts energy, and promotes longevity. Tryptophan is one of the essential amino acids in kefir that is well known for relaxing effect on the nervous system, and calcium and magnesium present in kefir are important minerals for a healthy nervous system. Kefir is also a good source of phosphorus, which is the second most abundant mineral in our bodies, helps one to utilize carbohydrates, fats, and proteins for cell growth, maintenance, and energy (Saloff-Coste, 1996). Kefir is a good diet for lactose-intolerant individuals who have the inability to digest significant amounts of lactose that is the predominant in milk. The lactose content is decreased in kefir and the galactosidase level is increased as a result of fermentation (Otles & Cagindi, 2003).

Kefir's antimicrobial activity, which has been studied through in vitro studies, is attributed to competition with pathogens for available nutrients, as well as the production of organic acids, bacteriocins, carbon dioxide, hydrogen peroxide, ethanol, and diacetyl. In vitro studies have shown that kefir exhibits antimicrobial activity against *Candida albicans*, *Salmonella typhi*, *Salmonella enterica*, *Shigella sonnei*, *Escherichia coli*, *Bacillus subtilis*, *Enterococcus faecalis*, and *Staphylococcus aureus*. Bioactive peptides produced from casein during fermentation have been shown to stimulate the immune system in animal models, while kefiran showed decreased ovalbumin-induced cytokine production in a murine asthma model (Dimidi et al., 2019). In vitro and animal studies have shown potential therapeutic effects like antioxidative, antihypertensive, anticarcinogenic, and cholesterol- and glucose-lowering effect of kefir.

3.2.3.4 Effects of kefir in gastrointestinal disorders

The effect of kefir and its microbial components in improving gut health has been widely investigated. Kefir or its constituent strains have been shown to have a considerable impact on the gut microbiota population with increases in *Lactobacillus*, *Lactococcus*, and *Bifidobacterium* concentrations, and reductions in *Proteobacteria* and *Enterobacteriaceae* concentrations, being demonstrated in numerous animal studies. In humans a study in 45 people with IBD showed that a kefir-specific strain, *L. kefiri*, was identified in most participants' feces 4 weeks following 800 mL/day kefir consumption, and a significant increase in total stool *Lactobacillus* abundance was found compared to control (no kefir) in patients with Crohn's disease.

Kefir is tolerated by people with lactose malabsorption as it contains β-galactosidase-expressing bacteria like *K. marxianus* that hydrolyses lactose. But despite its higher β-galactosidase content, a small crossover RCT in 15 people with lactose malabsorption showed that although kefir produced a significantly lower breath hydrogen concentration compared to milk, it was similar following kefir and plain yoghurt consumption. This is suggestive that kefir and plain yoghurt improved lactose digestion to a similar degree. It also reduces flatulence severity when compared to milk.

A double-blind RCT investigating the impact of 500 mL/day kefir, compared to 250 mL/day milk, on *H. pylori* eradication rates in patients with dyspepsia and diagnosed *H. pylori* infection who were treated with a triple antibiotic therapy for 2 weeks, showed that the rate of *H. pylori* eradication was significantly higher in the kefir group (78%) compared to the control group (50%; $P = .026$). The occurrence of diarrhea, abdominal pain, and nausea was also significantly reduced in the kefir group compared to control, suggesting kefir may be given as an additional therapy during treatment for *H. pylori* infections (Dimidi et al., 2019).

3.2.3.5 Antiinflammatory effect of kefir

Kefir has been proved to interact with several cellular pathways and regulate biological processes, including antioxidative process, apoptosis, and proliferation. Cancers have been mainly caused by dysregulation of immune system and immunomodulatory function. The bioactive compounds in kefir induce antiinflammatory response by the activation of macrophages and phagocytosis and nitric oxide (NO) production. This could improve the immunomodulatory mechanism and immune function. Increase in secretion of TNF-α and cytokines such as IL-5, IL-6, IL-1β, and IL-12 and decrease in secretion of IL-8 are caused by kefir consumption. High level of IL-5 and TNF-α will lead to high level of IgA secretion. The decreased level of IL-8 may control inflammatory response by suppressing chemotaxis and activation of neutrophils. Suppressed T helper 2 immune response and activated T helper 1 immune response induce antiallergic effect (Sharif et al., 2017).

3.2.3.6 Anticancer activity of kefir

In vitro studies have shown the role of kefir, having antitumor effects in different cancers. In 2007 a study performed by Chen et al. on effect of extracts of kefir on MCF-7 and HMECs cell lines demonstrated that kefir depressed MCF-7 cell growth in a dose-dependent manner. In an in vitro murine study of cytokines involved in the prevention of a murine experimental breast cancer by kefir, the modulatory capacity of kefir cell-free fraction on the immune response in mammary glands and tumors was demonstrated. According to previous studies, kefir extracts induce apoptosis, cell cycle arrest and reduce tumor growth in breast cancer cells.

In leukemia, kefir intake has caused increased apoptosis and decreased cell proliferation. Maalouf, Baydoun, and Rizk (2011) demonstrated a significant decrease in cell proliferation in human T lymphotropic virus type I-negative malignant T lymphocyte cell lines, in dose- and time-dependent manners. Kefir displayed an antiproliferative effect by upregulating TGF-β1 and downregulating TGF-α expression. The effects of kefir on skin cancer cells has been shown that kefir suppressed the morphological alterations caused in melanoma cell lines (HMV-1 and SK-MEL and TIG-1), caused by UVC (Ultraviolet C) irradiation. Kefir extract application after UVC irradiation of HVM-1 cells resulted in a notable reduction in intracellular reactive oxygen species (ROS), increased by exposure to UVC irradiation. The wound-healing properties of kefir have also being studied, although no proven evidence exists.

The assessment of the effect of *L. kefiri*, on gastric cancer cells (AGS) by Ghoneum and Felo, revealed apoptosis induction in AGS cell line. Apoptosis was associated with declined polarization of mitochondrial membrane potential (MMP) and reduced Bcl2 expression. Downregulation of Bcl-2 and decrease in the MMP in AGS cells may lead to the release of proapoptotic molecules and subsequently activation of caspases, causing apoptosis. The supernatants of kefir contain high amounts of acetic and lactic acids and showed meaningfully antioxidant capacity. The evidence proposes that kefir and ayran can decrease DNA damage, which might be due to their antioxidant capacities. Kefir anticancer potential property on CRC cell lines (Caco-2 and HT-29) exhibits the ability of kefir to induce cell cycle arrest at the G1 phase. In addition, kefir decreases the expression of TGF-β1 and TGF-α in HT-29 cell line. Upregulation of the Bax/Bcl-2 ratio supports the proapoptotic effect of kefir and increase of p53 independent-p21 expression (Sharif et al., 2017).

Thus we can ascertain that kefir is one of the best therapeutic functional foods and is likely to be recognized for effective treatment of various disorders, especially as an anticancer agent in the near future.

3.3 Cereal- and legume-based: idli, dosa, appam

Cereal-based fermented foods have gained importance more comparatively with dairy-based due to the presence of the good bacteria, vitamins, enzyme, etc. Moreover, multistrain probiotics may provide greater beneficial effects than the monostrain cultures. The growth of enteropathogens is prevented by the synergistic actions of the exogenous microbiota thereby creat-

ing a sociable environment for commensals (native colonizing organisms). As a result, these multistrain probiotics enhance digestibility, produce metabolites like short-chain fatty acid, butyrate, which have a positive effect on epithelial lining of the gastrointestinal tract, enhance mucosal cell differentiation, and this may also promote the immune barrier function of the epithelium, and on peristalsis, which improves transit (Charalampopoulos, Pandiella, & Webb, 2002). Cereal-based food components are a very good growth media/carriers for probiotics as it has an optimum buffering capacity to protect the organisms even in the harsh environment of the intestine (Lin, 2003). Thus by considering the beneficial effects, the grain-based fermented foods have now become more popular than the dairy-based foods (Saarela, Lahteenmaki, Crittenden, Salminen, & Mattila-Sandholm, 2002).

3.3.1 Rice-based food

Rice is the second most cereal crop belonging to the family *Oryza sativa.* It is the staple food for almost half of the world's population. In India, rice has gained an irreplaceable status as it has innumerable qualities (Umadevi, Pushpa, Sampathkumar, & Bhowmik, 2012).

In terms of area, production, and consumer preference, rice is the most important crop and India is the second largest producer and consumer of rice in the world (Gross & Zhao, 2014). Rice is converted and consumed in different forms such as flour, paste, laja (parched rice), batter, boiled, puffed, flaked, flattened, fried rice, and dried, and as sprouted seedlings (Mohan et al., 2014). Since rice is the most available and common food resource in the Indian subcontinent, preparation of different types of fermented foods and beverages from it is a regular practice and it plays an integral role in both culture and tradition (Roy, Kala, Farooquee, & Majila, 2004).

3.3.1.1 Idli

Idli is a soft rice cake consumed mostly in the Indian subcontinent (Blandino, Al-Asceri, Pandiella, Cantero, & Webb, 2003). This rice cake is highly nutritious and is consumed as breakfast in south India. The substrates are rice (*O. sativa*) and black gram dhal (*Phaseolus mungo*) which are being washed and soaked separately either overnight or 4–8 hours and then ground separately and mixed. The batter is kept overnight at room temperature for fermentation and then steamed to idli (Satish Kumar et al., 2013). Black gram (*P. mungo* L.) is the primary ingredient having a natural fermentation microflora and acts as the substrate for the fermentation of batter. Sometimes, sour buttermilk is also used as a source of starter (Radhakrishnamurty, Desikachar, Srinivasan, & Subrahmanyan, 1961). Leavening is the major process in fermentation in idli preparation.

3.3.1.1.1 Microorganisms Microorganisms involved in the leavening process are heterofermentative lactic acid bacterium *Lactobacillus mesenteroides* and homofermentative LAB *Streptococcus faecalis* regulate the acidity of batter (Mukherjee, Albury, Pederson, Vanveen, & Steinkraus, 1965). In addition to these, probiotic microorganisms such as *L. plantarum* and *Lactobacillus lactis* are also present in idli batter and produce vitamin B12 and β-galactosidase enzyme promoting health (Iyer, Singhal, & Ananthanarayan, 2013).

3.3.1.1.2 Nutritional value Idli contains about 3.4% protein, 20.3% carbohydrate, 70% moisture, 1% verbascose, 0.2% stachyose, and raffinose. Fermentation increases level of amylase, proteinase, total acids, batter volume, soluble solids, essential amino acids (lysine, cysteine, and methionine), nonprotein nitrogen, soluble vitamins (folate, vitamin A, vitamin B1, vitamin B2, and vitamin B12) content, with reduction in antinutrient phytic acid (Moktan, Roy, & Sarkar, 2011).

3.3.1.1.3 Therapeutic potential Idli is generally regarded as antiobesity as it has a low-calorific value and used in weight-losing diet. It is also a prescribed diet for people with risk of cardiovascular diseases, high blood pressure, and stroke and given as dietary supplement to treat children suffering from protein calorie malnutrition and kwashiorkor (Blandino et al., 2003; Purushothaman, Dhanapal, & Rangaswami, 1993).

3.3.1.2 Dosa

Dosa is a fermented recipe like idli, and the process of batter preparation and fermentation is same as idli except for the batter consistency. It is a thin and crispy dish consumed by every Indian (Steinkraus, 1996). Dosa fermentation increases the amount of total acids, total volume, total solids, nonprotein nitrogen, free amino acids, amylase, proteinases, vitamin B1, vitamin B2, folic acid, amino nitrogen, and antimicrobial and antioxidant substances. It reduces antinutrients and enhances the bioaccessibility of zinc and iron.

3.3.1.2.1 Microorganisms LAB like *Lactobacillus mesenteroides*, *S. faecalis*, *S. thermophilus*, *Lactobacillus fermentum*, *Bacillus amyloliquefaciens*, *Lactobacillus lactis*, *L. delbrueckii*, and *L. plantarum* are predominant groups that

participate in dosa fermentation. Minor groups related to yeasts belong to *S. cerevisiae*, *Debaryomyces hansenii*, *Trichosporon beigelii*, *Torulopsis* sp., and *Trichosporon pullulans* (Blandino et al., 2003; Satish Kumar et al., 2013).

3.3.1.2.2 Nutritional value of dosa One dosa contributes approximately 2.7 g of proteins, 18.8 g of carbohydrates, 1.1 g of fiber, 5.2 g of fat along with vitamin A 47.2 µg, vitamin B 0.1 mg, niacin 0.7 mg, folic acid 9.5 µg, calcium 10.9 mg, iron 0.5 mg, magnesium 22.4 mg, phosphorous 52.6 mg, sodium 3.3 mg, potassium 73.2 mg, and zinc 0.4 mg.

3.3.1.2.3 Therapeutic potential of dosa Dosa helps one to fight against prediabetic and postdiabetic conditions due to its low glycemic load and glycemic index and also provides adequate energy for prolonged physical endurance. It is believed that dosa has medicinal properties and can be used to increase fertility, weight of fetus, and breast milk and also considered as a remedy for rheumatism and neural disorders (Blandino et al., 2003; Gupta & Tiwari, 2014).

3.3.1.3 Appam

Appam is a rice-based and a very old conventional and traditional recipe. It is a popular food in Tamil Nadu and a regular food in Kerala states. The varying proportions of parboiled rice and black gram (*Vigna mungo*) are used for the preparation of appam in varying seasons. Conventionally, parboiled rice and urad dal paste are mixed to prepare the batter that is fermented overnight. The fermented batter is next mixed with sugar, grated coconut, or other seasonings. The final mixture is poured into an earthen mold or flat plates, covered, and cooked in low heat to obtain traditional appam in Kerala. In Tamil Nadu, it is cooked in the appam pan and served with coconut milk. Appam fermentation is acidic in nature like idli and dosa, due to the lactic acid producing bacteria. The details of microbial composition in the batter are yet to be explored.

3.3.1.3.1 Nutritional value of appam Appam can give 138.8 Cal, 3.7 g total fat, 0.1 g unsaturated fatty acids, 31.7 mg sodium, 13.5 mg potassium, 23.2 g total carbohydrate, 1.1 g dietary fiber, and 2.1 g protein, with vitamin A, B-complex, calcium, folate, iron, niacin, riboflavin, and thiamine. This is a healthy and easy digestible, nutritionally enriched food (Ray, Ghosh, Singh, & Mondal, 2016).

3.3.1.3.2 Therapeutic potential of appam Appam is a low-calorie food and helps in maintaining weight. It also reduces the risk of cardiac stroke as it lowers the cholesterol and fat deposits in the body. In olden days, toddy or kallu the neighborhood palm wine was used as a starter culture to ferment the appam batter, which enhances the taste (Yuvaraj, 2019).

3.4 Legume-based: tempeh, miso

3.4.1 Tempeh and miso

Fermented soy products have their origin in China and Japan, including fermented black soybean and red fermented tofu. There are many fermented soybean products from different parts of Asia, including tempeh, natto, miso, sufu, douche, soy sauce, and doenjang.

According to Dimidi et al. (2019), soybeans and rice are the most popular ingredients used in the preparation of legume- and cereal-based fermented foods. Asian countries developed their own methods to make fermented soybean products. The typical fermented soybean foods are consumed in Asian countries such as Korea, China, Japan, Indonesia, and Vietnam (Kwon et al., 2009). The most common Korean fermented soybean foods include doenjang, kanjang (soy sauce), kochujang, and chungkukjang. Originally natto and miso were Japanese versions of chungkukjang and doenjang, respectively. China also has various fermented soybean products such as doubanjiang, douche (sweet noodle sauce), tauchu (yellow soybean paste), and dajiang.

Legumes like soybean were first cultivated in Eastern Asia thousands of years ago and have long been important protein sources along with cereal proteins, in Asian countries. Soy-based foods have been a part of the staple diet, in Asia. The traditional soy-based food products are healthier compared to the processed soy foods because of their nutritive value and nutrient bioavailability. Consumption of soy-based products has been incorporated in the western diet only in the recent past. The average intake of soy protein in Asia ranges from 10–50 g/day compared to 1–3 g/day consumed by Americans (Barnes, Coward, Kirk, & Sfakianos, 1998). Along with essential nutrients, soybean products, especially the fermented soybean products, contain various functional components, including peptides, isoflavonoids, and more (Davis et al., 2007).

3.4.1.1 Preparation of miso and tempeh

Tempeh is a dish of fermented whole soybeans that originated in Indonesia but is now equally popular in Malaysia. Tempeh is a cake made of cooked and fermented soybeans held together by the fungal mycelium of *Rhizopus oligosporus*. The

process involves soaking soybeans overnight, boiling them with the hulls (Malaysian) or without the hulls (Indonesian) for half an hour. After the excess water is drained off, the beans are placed on a tray for inoculation with *R. oligosporus* or with a piece of tempeh as a starter culture. The fermentation is carried out at room temperature for 1–2 days or at 30°C–32°C for 20 hours. During fermentation, white mold mycelium covers the beans and binds them together into a solid sheet. The tempeh sheet is then cut into smaller pieces and sold. If it is to be stored for future use, it is usually blanched, sun dried, or frozen. Tempeh is usually cooked before it is eaten. Preparation usually involves frying, deep frying, or baking the product. It is also added to soups and fast foods and is used as a meat replacement in main dishes (Erickson, 1995).

Miso is a traditional Japanese paste of fermented soybean used for making soup. Miso is produced by fermenting soybeans with "Koji," produced from a mold *Aspergillus oryzae*. *S. cerevisiae* and LAB are sometimes additionally used. The thick paste obtained after fermentation is used for various food preparations like soup, spread, and stock consumed along with rice. Miso production varies greatly in terms of ingredients, temperature and fermentation time, salt concentration, and the strain of *A. oryzae* used (Dimidi et al., 2019).

Doenjang or Japanese miso is prepared by cooking the soybean and fermenting it for 1 or 2 months with Koji, like as solid fermentation under the outdoor roof of the Korea traditional house in winter and the paste is aged for longer periods.

3.4.1.2 Nutritional value and microbes

Cereals and legumes have been important sources of carbohydrates and proteins. They have been widely consumed in various fermented forms in different parts of the world. These foods contain many bioactive compounds that are absent in unfermented foods. The fermentation process in soy removes trypsin inhibitors found on the coating of soy that interfere with the absorption of nutritive compounds, thus promoting enhanced nutritive bioavailability fermenting or culturing soy has also been shown to enhance the bioavailability of iron and copper and to render these nutrients in their most beneficial forms. These products cannot be compared to uncultured soy foods like tofu, soymilk, soy nuts, and soy protein isolates which have potentially antinutritive value due to their high oxalic acid levels and can block the absorption of essential nutrients such as calcium. Isoflavones are flavonoid compounds that are produced by plants belonging mainly to the Fabaceae family. Main sources of dietary isoflavones are soybeans and red clover (*Trifolium pratense*). Other plants with a high content of isoflavones are mung beans (*Vigna radiata*), kudzu (*Pueraria lobata*), lupine (*Lupinus* spp.), fava bean (*Vicia faba*), psoralea (*Psoralea corylifolia*), poinciana (*Caesalpinia pulcherrima*), and alfalfa (*Medicago sativa*). The fermentation process also converts the isoflavone precursors genistin and daidzin to their active isoflavone forms, genistein, and daidzein (Danciu et al., 2017).

The microbial composition of "tempeh" varies according to variations in production. Tempeh contains LAB, *Enterococcus faecium*, and *Rhizopus* filamentous fungi. Fermentation of soybeans has been shown to reduce concentrations of protease inhibitors, phytic acid, and phenols, antinutritional factors that are high in raw soybeans, which may relate to phytases expressed by *Rhizopus* species in tempeh.

A. oryzae is the major microorganism in the final product of "miso" when it is made in the traditional method. A microbial analysis following the start of fermentation revealed the presence of *B. subtilis*, *B. amyloliquefaciens*, *Staphylococcus gallinarum*, and *Staphylococcus kloosii*, but only the *Bacillus* species remain in the final product. A range of miso samples have also been shown to contain *Lactococcus* species GM005 that produces a bacteriocin with strong antibacterial activity that inhibits other bacteria (Dimidi et al., 2019).

3.4.1.3 Therapeutic potential

3.4.1.3.1 Role in gut health Fermented soy products have many beneficial effects on health, including alleged "anticarcinogenic," "antidiabetic," "antioxidant," "antiinflammatory," and "antihyperlipidaemic" effects, although much of the existing evidence is limited to in vitro and animal studies.

Tempeh produced huge changes in the human microbiota in an in vitro gut simulator model, increasing the *Bifidobacterium*, *Lactobacillus*, *E. coli*, and *Enterococcus* abundance. In an open-label uncontrolled study of 10 healthy human volunteers, tempeh consumption led to greater stool *Akkermansia muciniphila* abundance and immunoglobulin A concentrations, suggesting that tempeh consumption influences gut microbiota in humans. However, a larger, controlled study is needed to establish the effects of tempeh on gastrointestinal microbiota composition.

In Sprague–Dawley rats, stool *Bacteroidetes*, *Firmicutes*, *Clostridium leptum*, and *Bacteroides fragilis* loads increased following tempeh supplementation compared to rats fed nonfermented soybeans (Dimidi et al., 2019).

A cross-sectional study showed that miso soup intake was inversely proportional to subjective gastroesophageal reflux disease, functional dyspepsia, and reflux scores when adjusted for other dietary factors. This association was hypothesized to relate to histidine, glutamate, and aspartate found in miso soup. But no animal or human studies have investigated this system to date (Yang, Park, Pak, Chung, & Kwon, 2011).

3.4.1.4 Antioxidant potential

Tempeh has been associated in vitro with greater free-radical and superoxide scavenging ability than unfermented soybeans, due to changes in polyphenol content and digestibility in soybeans following fermentation. But to date, there are no randomized control trials of the effects of tempeh consumption in humans. The health effects listed earlier require further controlled investigations and human trials.

3.4.1.5 Therapeutic potential of soy isoflavones

The therapeutic activities of soy isoflavones were evaluated for their estrogenic, lipid-lowering, antidiabetic, antiinflammatory, cardioprotective, or anticancer effects. In vitro and in vivo studies were performed in order to evaluate the therapeutic potential and the mechanisms of action of these compounds. The high concentrations of isoflavones "genistein" and "daidzein" are found in soybeans. Genistein is structurally similar to estrogen and may influence breast cancer risk through estrogen receptor binding, which has been demonstrated in vitro. Further in vitro studies have demonstrated that genistein may reduce cancer risk by promoting cell-cycle arrest inducing apoptosis and reducing cancer cell migration.

Genistein and daidzein are higher in fermented soybean products (miso and natto) compared to unfermented products such as soy milk and tofu. There are currently no RCTs investigating the effects of miso in functional bowel disorders (Danciu et al., 2017). There is therefore limited evidence for the effects of miso on gastrointestinal conditions and microbiota. There are several observational studies demonstrating associations between miso intake and the risk of stomach cancer; however, the strength and direction of these associations remain unclear.

3.5 Vegetable- and fruit-based: kombucha, pickles, kimchi, and sauerkraut

3.5.1 Kombucha

Kombucha or "tea fungus" is a sweetened fermented black or green tea that uses bacteria for fermentation. It is obtained from the fermentation of tea, mainly black tea, but other varieties that are commonly used as a base for its preparation are green tea, oolong tea, also known as blue tea (Watawana, Jayawardena, Gunawardhana, & Waisundara, 2015), and also an infusion of rooibos leaves (*Aspalathus linearis*) (Gaggia et al., 2018) with added sugar as a substrate for fermentation. Kombucha first originated in northeast China about 220 BCE and later was used in Japan in CE 414 as a medicine and spread through trade routes to Russia and Eastern Europe (Watawana et al., 2015; Dufresne & Farnworth, 2000). "Kombucha" is the commonly used name for the beverage on a commercial basis, but it also goes by other popular names such as Chainii grib, Chainii kvass, Champignon de longue vie, Ling zhi, kocha kinoko, and red tea fungus (Malbaša, Lončar, Vitas, & Čanadanović-Brunet, 2011).

Although fermented foods have been used internationally for thousands of years, kombucha has only recently become popular in many parts of the world, including the United States. Kombucha's worldwide popularity has grown since World War II (Jayabalan, Malbasa, Loncar, Vitas, & Sathishkumar, 2014) PepsiCo purchased KeVita, a popular functional probiotic and kombucha beverage maker in 2016 after recognizing its market value and potential. The retail sales of kombucha have increased considerably in the last 3 years and are one of the fast-growing functional food beverages (Troitino, 2018; Watson, 2018). The bacteria turn the sugar in the tea to alcohol. As a result, kombucha contains a low level of alcohol but not enough to cause intoxication. So it is one of the low-alcoholic, fermented beverages in the world (Baschali, Tsakalidou, Kyriacou, Karavasiloglou, & Matalas, 2017).

3.5.1.1 Kombucha preparation

Kombucha is made by fermenting black tea or sometimes green and oolong tea and sugar, with a symbiotic culture of bacteria and yeast (SCOBY) (Leal, Suarez, Jayabalan, Oros, & Escalante-Aburto, 2018), generally for 7–10 days. The SCOBY is nothing but a biofilm of microorganisms that looks like a mushroom cap, forming a floating cellulose layer on the surface of the fermented tea. The longer the fermentation, the thicker the layer becomes. It becomes a starter culture for subsequent use in concoctions.

The typical production of kombucha is carried out using 5 g of tea leaves per liter of water to which sugar is added, which will serve as a substrate for tea fermenting bacteria and yeasts. Approximately 50 g of sugar per liter of water is used. Before adding the SCOBY or the starter culture which can be a bit of already prepared kombucha, the beverage should be at a temperature close to 20°C. Clean, sanitized utensils should be used and work must be carried out in clean areas while making kombucha, in order to have control over the growth of microorganisms and to prevent unwanted contamination (Watawana et al., 2015).

The fermentation should be stopped when the titratable acidity reaches 4.0–4.5 g/L—a level which is accepted by long-time consumers of the kombucha beverage and is known as the optimal consumable acidity (Reiss, 1994). Control of

pH is also another important factor. The process is generally stopped when a pH of 4.2 is reached in order to prevent the overproduction of acetic acid (Kovacevic et al., 2014). The final product is pasteurized to prevent the overproduction of alcohol and carbon dioxide, 0.1% of sodium benzoate and 0.1% of potassium sorbate are added as food preservatives, and finally it is refrigerated (Watawana et al., 2015). Kombucha fermentation period optimally requires a minimum of 3 days to a maximum of 60 days, depending on cultural practices (Watawana et al., 2015). The fermentation of kombucha is usually carried out at room temperature, by optimizing fermentation time. A SCOBY or the resulting liquid at a 10% concentration from a previous fermentation is used for fermentation.

3.5.1.2 The beneficial microbes present in kombucha

In kombucha the most abundant bacteria in the symbiotic culture belong to the *Acetobacter* and *Gluconobacter* bacteria genus (Jayabalan et al., 2014). Belonging to the Acetobacteraceae family, these bacteria are Gram-negative aerobic bacilli (Stasiak & Blazejak, 2009). They have the ability to oxidize the acetate anion in carbon dioxide. The strains of the genus *Acetobacter* produce acetic acid from ethanol. This process is carried out by two main enzymes alcohol dehydrogenase and aldehyde dehydrogenase that produce acetic acid, which enters the Krebs cycle obtaining water and carbon dioxide as end products (Teyssier & Hamdouche, 2016). The genus *Gluconobacter* is not capable of oxidizing acetate through the Krebs cycle, since it lacks the enzymes like succinate dehydrogenase and alpha-ketoglutarate dehydrogenase, necessary for the oxidation process, leading to accumulation of products, like gluconate, in the medium (Zoecklein, Fugeslang, Gump, & Nury, 1999). Moreover, different yeast species can be found in kombucha, which are greater acetic acid bacteria (AAB) (Jayabalan, Malini, Sathishkumar, Swaminathan, & Yun, 2010).

The SCOBY comprises a complex mixture of bacteria and yeasts that include various AAB (e.g., *Acetobacter xylinum*, *Acetobacter aceti*, *Acetobacter pasteurianus*, and *Gluconobacter oxydans*) and yeasts (e.g., *Saccharomyces* species, *Zygosaccharomyces kombuchaensis*, *Torulopsis species*, *Pichia species*, *Brettanomyces species,* and *Zygosaccharomyces bailii*) (Watawana et al., 2015; Greenwalt, Steinkraus, & Ledford, 2000; Vina, Semjonovs, Linde, & Deninja, 2014). Several LAB have also been isolated. After fermentation, kombucha becomes a combination of chemical components (Miranda, Lawton, Tachibana, Swartz, & Hall, 2016; Kaczmarczyk & Lochynski, 2014), including sugars; tea polyphenols; organic food acids; fiber; ethanol; amino acids, including lysine; essential elements such as Cu, Fe, Mn, Ni, and Zn; water-soluble vitamins such as vitamin C, and several B vitamins; carbon dioxide; antibiotic substances; and hydrolytic enzymes (Jayabalan et al., 2014).

3.5.1.3 The health benefits of tea

Tea is made up of various components, like caffeine, alkaloids, amino acids, carbohydrates, proteins, chlorophyll, fluoride, aluminum, minerals, and trace elements (National Cancer Institute, 2010). The flavonol or catechin content is very high in fresh tea leaves. These flavonols and flavonoid derivatives are characterized by their monomeric structure, including epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) (Valenzuela, 2004).

The oxidation process of black tea induces a change in the monomeric structure of catechins, resulting in dimeric and polymeric flavonols, known as theaflavins and thearubigins. The content of theaflavins and thearubigins is lower in green tea because it does not go through a fermentation process. For this reason, teas have a varied effect on health due to their different composition (Figure 29.1 Main factors affecting probiotics' viability in foods. gr1).

3.5.1.4 The nutritive value and therapeutic potential of kombucha

The widely growing popularity of kombucha is because of its acclaimed health and nutritional benefits such as antiinflammatory and antioxidant properties (Jayabalan, Subathradevi, Marimuthu, Sathishkumar, & Swaminathan, 2008). It is also believed to promote immune system health and could also reverse metabolic disorders. The effect of kombucha in reduction of cholesterol levels and blood pressure, reduction of cancer propagation, the improvement of liver, and gastrointestinal functions have also been explored widely (Leal et al., 2018), although direct evidence for the use of kombucha in human health has not been proved through clinical trials on human subjects and empirical research testing the effects of kombucha on any human health or health-related outcome has not been reported according to a wide review of 300 research articles published between 1994 and 2017. The positive evidence of the benefits of kombucha in human health is difficult to show because of the difficulty to organize randomized and controlled intervention trials, which are also very expensive. However, several studies reported a wide spectrum of beneficial activities, by studies in vitro or in animal models, including antimicrobial activity against food borne and human pathogens, hepatic detoxification in rats, antiinflammatory, hypocholesterolemic, antiproliferative, and hypoglycemic activities (Jayabalan et al., 2014; Villarreal-Soto, Beaufort, Bouajila, Souchard, & Taillandier, 2018). To date, only one study has been applied in human subjects' research, examining the health benefits of kombucha (Hiremath, Vaidehi, & Mushtari, 2002).

3.5.1.5 Antimicrobial and antioxidant potential

Chemical assays of kombucha beverage signify the presence of a variety of compounds, including organic acids, mainly acetic, gluconic, and glucuronic acid (GlcUA), although citric, L-lactic, malic, tartaric, malonic, oxalic, succinic, pyruvic, and usnic acids may also be found; sugars (sucrose, glucose, and fructose), water-soluble vitamins (B1, B2, B6, B12, C), amino acids, biogenic amines, purines, pigments, lipids, proteins, hydrolytic enzymes, ethanol, AAB and LAB, carbon dioxide, polyphenols, minerals (manganese, iron, nickel, copper, zinc, plumb, cobalt, chromium, and cadmium), anions (fluoride, chloride, bromide, iodide, nitrate, phosphate, and sulfate), D-saccharic acid-1,4-lactone (DSL), and metabolic products of yeasts and bacteria (Jayabalan et al., 2010, 2014). The significance and quantity of the chemical components are variable, depending on the microorganisms of the symbiotic culture used for fermentation of kombucha. These substances act as potent antioxidants and protect against the development of diseases. These have been studied mainly in vitro, while others are based on clinical and epidemiological evidence (Valenzuela, 2004).

3.5.1.6 Detoxification potential

Kombucha is a high source of glucuronic acid, which has a detoxifying effect against drug, bilirubin, and chemicals, as well as pollutants and excess of steroid hormones (Nguyen, Nguyen, Nguyen, & Le, 2015). It has also been shown to improve in vitro antimicrobial activity and improve sleep (Greenwalt et al., 2000; Sreeramulu, Zhu, & Knol, 2000).

3.5.1.7 Potential for cardiovascular disease prevention

French epidemiologists in the 1980s concluded that in France, where there was a high intake of dietary cholesterol and saturated fat and daily consumption of wine, low cardiovascular heart disease (CHD) death rates were observed. This French paradox states that moderate alcohol consumption has a protective effect against CHD, since it raises HDL cholesterol concentrations (Ferrières, 2004). Since the daily consumption of ethanol in a lower concentration can protect the human body from cardiovascular diseases, the consumption of kombucha, which has a low concentration of ethanol, could also have a role in preventing cardiovascular diseases.

3.5.1.8 Antidiabetic and anticancer potential

Kombucha may help one to stabilize blood sugar levels and aid in the management of diabetes. Kombucha may also be helpful in managing type II diabetes. A 2012 study found that kombucha helped to manage blood sugar levels in rats with diabetes. This finding suggests it may be helpful in type II diabetes management. Again, more research is needed to say with certainty whether kombucha can have the same benefits in type II diabetes management for humans. There is now a prevalent study of the effects of kombucha against certain types of cancers. In vitro studies carried out in 2008 and 2013 have suggested that kombucha potentially decreased the survival rate of cancer cells. But studies have not proved so far that people who drink kombucha regularly are less prone to developing cancer.

3.5.1.9 Potential in improving mental health and stability

Consuming kombucha has been found to be beneficial in alleviating depression. There could be a link between probiotics and depression, and drinking probiotic-rich kombucha could help one to promote positive mental health. There are strong links between depression and inflammation, so the antiinflammatory effect of kombucha may help one to alleviate some of the symptoms of depression. However, further research is needed to prove how effective they are.

3.5.1.10 Risks involved in kombucha consumption

Although kombucha has many potential health benefits, it is of great importance to be careful when making kombucha at home, as it can ferment for too long. It is also possible for kombucha to become contaminated when not made in a sterile environment. Overfermentation or contamination may cause health problems, so it may be a safer option to buy kombucha in a store than to make it at home. Store-bought kombucha normally has lower alcohol content than homemade versions, but it is important to check the sugar content. There are many potential health benefits of kombucha. However, it is important to remember that research is ongoing and not all benefits have been proven in studies with human participants. If made properly or bought in-store, kombucha is a probiotic-rich drink that is safe to enjoy as part of a healthful diet and it does hold therapeutic potentials too, which may be proven soon.

3.5.2 Pickles

Pickling is an ancient gastronomic craft (predate to 2400 BCE) of preserving food in brine and/or vinegar. Multiple ancient civilizations, such as ancient Egyptians, Chinese, and Indians, used pickling for food preservation (Chakraborty &

Roy, 2018). The fermentation process for vegetables can result in nutritious foods that may be stored for extended periods, 1 year or more, without refrigeration (Nguyen & Carlin, 1994). It is the process of conversion of sugars to acids by microorganisms (Nurul & Asmah, 2012). Pickles are good appetizers, consumed by people of all ages and contain large amounts of lactobacilli that are important for the digestion of grains and vegetables and have beneficial probiotic properties (Lal, Siddapp, & Tandon, 2010). Pickles that are prepared by LAB fermentation have unique flavor and beneficial health effects (Choi et al., 2013). LAB in pickles help one to enhance human nutrition by providing vitamins, minerals, and carbohydrates and also produce various aroma components, bacteriocins, and exopolysaccharides. These metabolic products impart some characteristic properties such as taste, texture, and longer shelf life to the products (Leroy & de Vuyst, 2004). LAB carry out detoxification of toxic compounds and degradation of mycotoxins in specific cases and therefore can reduce the health risk. A variety of ingredients such as salt, vinegar, spices, namely, chilli powder, turmeric powder, fenugreek seed, asafetida, sugar, jaggery, and oil, are frequently used in pickling in different parts of the world (Monika, Kumari, Kunzes, & Bhalla, 2016). The microorganisms involved in the fermentation of pickles have been identified as *L. plantarum*, *L. brevis*, *Le. mesenteroides* and *Pediococcus cerevisiae*, *Pediococcus pentosaceus* and *E. faecalis* (Choi et al., 2013).

Acid-fermented vegetables are produced in different regions of the world and are used as salad and side dish (Lee, 1994). Sauerkraut is produced in Germany with the major ingredients cabbage, salt, and the microorganisms present in it are *La. mesenteroides*, *L. brevis*, and *L. plantarum.* Kimchi is produced in Korea with Korean cabbage, radish, various vegetables, salt, and the microorganisms present in it are *Lactobacillus mesenteroides*, *L. brevis*, and *L. plantarum.* Dhamuoi is produced in Vietnam with cabbage, various vegetables, and the microorganisms present in it are *Lactobacillus mesenteroides* and *L. plantarum.* Dakguadong is produced in Thailand with mustard leaf, salt and the microorganisms present in it are *L. plantarum* salad, whereas side dish Burong mustasa is produced in Philippines with mustard and the microorganisms present in it are *L. brevis* and *P. cerevisiae* (Lee, 1994).

3.5.2.1 Biochemistry of vegetable fermentations

Fermentation is by definition an anaerobic process. During the fermentation of cucumbers, cabbage, and olives, glucose and fructose are converted to lactic acid, acetic acid, ethanol, and CO_2 by LAB and yeasts (Gottschalk, 1986). Cucumber (*Cucumis sativus* L.) is a member of the Cucurbitaceae family and a popular vegetable crop used in India as an ancient traditional medicine (Mukherjee, Nema, Maity, & Sakar, 2013). Cucumbers were first fermented around 2000 BCE in the Middle East. Early-written records of cucumber pickles come from paper fragment remains of a play (*The Taxiarchs*) by the Greek writer Eupolis (429–412 BCE), and pickles are also mentioned several times in the Christian Bible (Lee, 2001). The word "pickle" by itself usually refers to a pickled cucumber. The primary retail fermented vegetable products produced in the United States and Europe are cucumber pickles, olives, and sauerkraut. In Asia a variety of fermented vegetable products are available, including pickles and fermented cabbage, notably kimchi in South Korea (Fleming, Kyung, & Breidt, 1995). Other cucumber pickles include deli-style, half-sour cucumber pickles, which are partially fermented prior to consumption (Etchells, Bell, & Moore, 1976).

3.5.2.2 Cucumber fermentations

In the United States, commercial cucumber (*C. sativus*) fermentations are commonly done in 30,000–40,000-L, open-top, fiberglass tanks that are located out-of-doors so the brine surface is exposed to sunlight.

The UV radiation in sunlight is relied upon to kill aerobic surface yeasts that can metabolize lactic acid produced by the fermentation. Cucumbers are covered with salt brine and held below the brine surface with wooden headboards. Fermentations are typically carried out in brine equilibrated at about 6% NaCl. Calcium chloride (0.1%–0.4%, equilibrated) is added to the cover brine to maintain the firm, crisp texture of the fermented cucumbers during fermentation and storage (Breidt, McFeeters, Perez-Diaz, & Lee, 2013). Cucumber fermentations typically undergo a homolactic acid fermentation, which does not result in the production of carbon dioxide from sugars (glucose and fructose, about 1% each). However, carbon dioxide may be generated from the respiration of cucumbers and by the decarboxylation of malate during the initiation of fermentation (McFeeters, Fleming, & Thompson, 1982.).

3.5.2.3 Olive fermentations

Like cucumber and cabbage fermentations, olive fermentation practices are based on traditional methods that have been modified for large-scale commercial production. There are several methods used for processing olives (Fernandez, Garcia, & Balbuena, 1995).

3.5.2.4 Lingri pickle

Lingri is a fern locally available in high-altitude areas of Himachal Pradesh. Its pickle is prepared during summer when there is ample availability of this fern (Kapoor, Kanwar, & Gupta, 2010). Rhizome of lingri is used in the treatment of cough, asthma, fever, dyspepsia, stomach-ache, and diarrhea, and its leaves are useful in constipation and used as antibacterial (Semwal, Kaushik, Bhatt, & Negi, 2011).

3.5.2.5 Kachnar pickle

Kachnar (*Bauhinia variegata* L.) is a good source of nutrients especially in fiber component and can be beneficial for the patients with constipation and cardiovascular diseases. Bark is used in removal of intestinal worms (Yadava & Reddy, 2003).

3.5.2.6 Beedana pickle

Beedana (*Cydonia oblonga* Mill.) is an edible pear-shaped fruit mostly grown in Kullu district of Himachal Pradesh. Consumption of beedana fruit is considered to help in curing the people suffering from tuberculosis, hepatic insufficiency, diarrhea, dysentery, gastric ulcer, liver, and eye diseases.

3.5.2.7 Lasura pickle

Lasura (*Cordia myxa* L.) is green-colored fruit containing gum-like sticky material and consumed by the rural people of Mandi, Bilaspur, Kangra, Una, and Hamirpur districts in Himachal Pradesh as vegetable curry and as pickle. Lasura is considered to be helpful in decreasing the blood pressure and also consumed as antidiabetic.

3.5.2.8 Nashpati (pear) pickle

Pear pickle (*Pyrus communis* L.) is prepared in Kullu district of Himachal Pradesh during the month of July and August. Pears are a good source of dietary fiber, antioxidant, minerals, and good quantity of vitamin C.

3.5.2.9 Chukh

Chukh (*Capsicum annuum* L.) is a traditional fermented delicacy of Chamba district of Himachal Pradesh. It is a pickle made of chillies, lime juice, salt, and mustard.

3.5.2.10 Tamatar pickle

Tomatoes (*Solanum lycopersicum* L.) are the source of vitamin A, C and used in salads, sauces, flavoring in soups and prepared as pickles. Tomatoes are considered to promote gastric secretions and stimulate liver and also useful in asthma, bronchitis, and dyspepsia.

3.5.2.11 Dheu pickle

Dheu (*Artocarpus lakoocha* Roxb.) fruit has a pleasant unusual flavor and distinct taste which is not found in any other fruits and pickle. The fruits of dheu are useful for the treatment of dysentery, arthritic swelling, and skin diseases. Dheu pickle is an indigenous fermented product of Shimla, Mandi, and Hamirpur districts of Himachal Pradesh.

3.5.2.12 Elon pickle

Elon (*Cassia tora* L.) is distributed throughout India and known as Charota. Elon is used in Ayurvedic medicine as a treatment for leprosy, ringworm, and cardiac disorders. The herb eases the itchy skin when it is mixed with lime juice.

3.5.2.13 Kachalu pickle

Kachalu [*Colocasia esculenta* (L.) Schott] is a tuberous perennial with a group of underground corms consisting of a central large one and surrounding ones of varying sizes. The juice of the corm is laxative, demulcent, anodyne and is useful in hemorrhoids and congestion of the portal system (Monika et al., 2016).

3.5.3 Kimchi

Kimchi is a traditional fermented food of Korea that has been made for thousands of years (Lee, 1975). It was originally developed as a method that was used for preserving food for the winter. Kimchi gained popularity as a functional food

because of the phytochemicals formed during the fermentation process of kimchi (Park, Kil, Jung, Kong, & Lee, 2006). Kimchi is a broadly used term for salted and fermented vegetables consumed in Korea since CE 3rd or 4th century, based on the ancient records of kimchi preparation and consumption. The word "kimchi" originated from "chimchae," the Chinese character, meaning salted vegetables and it was called "dimchae," "dimchi," which ultimately evolved as "kimchi." The lactic acid (LA) fermentation of vegetables and fruits is a common practice for the improvement of nutritional and sensory features of food products (Demir, Bahceci, & Acar, 2006; Di Cagno, Coda, Angelis, & Gobbetti, 2013). The most popular kimchi in Korea is the baechu kimchi that is made primarily from Chinese cabbage (Cheigh & Park, 1994), although almost all vegetables cultivated in Korea have been used in kimchi preparation (Park et al., 2006). Other ingredients that are commonly added are red pepper powder, ginger, garlic, and radish. Ingredients like green onions, Chinese leeks, onions, pine nuts, gingko nuts and sugar and fish sauce made from shrimps, oysters, Alaskan pollack, squid, flounder, or yellow corvine are also added in variable amounts as seasoning ingredients (Cheigh & Park, 1994). The taste differs on the basis of the ingredients used, the fermentation conditions, and the LAB involved.

3.5.3.1 Kimchi preparation

The main ingredient of traditional kimchi is the Chinese baechu cabbage, or napa cabbage, which is cut and soaked in a 10%–15% salt solution for several hours until the leaves and stems become soft. The salted cabbage is then washed under running tap water and thoroughly drained. The seasonings are prepared, chopped, and then stuffed between the drained cabbage leaves. The stuffed Chinese cabbage is then packed into a traditional ceramic jar with a lid and stored in a cool place. Traditionally, this jar was buried in the ground for days to months depending on the environmental temperature. Kimchi is processed in a traditional ceramic jar called an "onggi" and it is stored in special refrigerators that can control the optimum temperature of the fermentation (Lee, Kim, Cho, & Kim, 2008). Kimchi fermented in an onggi has been shown to have higher nutritional profiles than when it was fermented in polyethylene, polypropylene, stainless steel, or glass containers (Jeong et al., 2011). The standard composition of baechu kimchi ingredients is as follows: the brined baechu cabbage (100%) is mixed with 13% sliced radish, 3.5% red pepper powder, 1.4% garlic, 0.6% ginger, 2.2% fermented anchovy juice, 1.0% sugar, 2% green onion, and a final salt level of 2.5%. A low temperature (5°C) is ideal for preparing a good-tasting product.

3.5.3.2 Kimchi fermentation and microbes

The microorganisms in kimchi were first investigated in Korea in 1939, and many LAB have since been isolated from kimchi. Systemic taxonomic studies have also been reported. Kimchi fermentation is separated into four stages based on acidity as initial stage (acidity <0.2), immature stage (acidity 0.2–0.4), optimum-ripening stage (acidity 0.4–0.9), and overripening or rancid stage (acidity >0.9) (Codex, 2001). The microorganisms that exist fermented kimchi are mostly lactic acid–producing bacteria and yeasts (Cheigh & Park, 1994). Kimchi contains very high levels of lactic acid–producing bacteria (108–109 CFU/g) (Chun, Kim, & Woo, 1999) such as *Le. mesenteroides*, *L. brevis*, *L. plantarum*, *P. cerevisiae*, *S. faecalis*, *E. faecalis*, *Pediococcus pentosaceus*, *Weissella koreensis*, and *Lactobacilli* species (Mheen & Kwon, 1984; Han, Lim, & Park, 1990). *Le. mesenteroides* and *L. plantarum* are found in the largest numbers. *Leuconostoc citreum*, *Leuconostoc gasicomitatum*, *Leuconostoc gelidum*, *Lab. sakei*, and *L. brevis* have been detected in kimchi samples using molecular methods. Studies reported that *W. koreensis* was the predominant species in commercial kimchi as identified by culture-independent methods (Kim & Chun, 2005). The aerobic bacteria and fungi are reduced during kimchi fermentation and yeasts increase under the low-temperature conditions (Mheen & Kwon, 1984). The crucial factor in determining the balance of microbial populations during fermentation is the temperature (Cho et al., 2006). Apart from this, a range of other important factors have been identified as optimum levels for consumption such as the final acidity which should fall between 0.6% and 0.7% and the pH which should be between 4.2 and 4.3 (Mheen & Kwon, 1984; Lee, 2009; Park & Rhee, 2005). The LAB profile during kimchi fermentation varies with pH and acidity.

Microbial communities of 13 kinds of commercial Korean kimchis at pH 4.2–4.4 (optimally ripened kimchi) using the pyrosequencing method showed the following percentage of microbes. *W. koreensis* (27.2%) accounted for the highest proportion among total LAB in kimchis, followed by *Lactobacillus sakei* (14.7%), *Weissella cibaria* (8.7%), *Lactobacillus graminis* (13.8%), *Lactobacillus gelidum* (6.3%), *Le. mesenteroides* (7.8%), *Leuconostoc inhae* (1.2%), *L. gasicomitatum* (1.2%), *Weissella confusa* (0.3%), *Leuconostoc kimchii* (0.3%), and other various *Lactobacilli* (3.3%), *Leuconostoc* (4.9%), and *Weissella* (8.1%) species followed.

3.5.3.3 The nutritional value and therapeutic potential of kimchi

Health Magazine in 2006 reported kimchi as one of the world's five healthiest foods (Lee, Lee, & Lee, 2012; Park, Jeong, Lee, & Daily, 2014; Dharaneedharan & Heo, 2016; Tamang, Watanabe, & Holzapfel, 2016). These health benefits result from the utilization of raw materials of high nutritive value and the microbiota taking part in the fermentation. Vegetables

belonging to the Brassicaceae family have been reported to contain a number of compounds with health-promoting potential, including dietary fibers, minerals, amino acids, vitamins, carotenoids, glucosinolates, and polyphenols.

The nutritive value of Chinese cabbage [*Brassica rapa* L. *pekinensis* (Lour.) Olsson] is attributed to the presence of vitamins A and C, 34 amino acids, including threonine, arginine, γ-aminobutyric acid, alanine, asparagine, serine, and glutamic acid, being abundant, 10 mineral elements among which Ca, Mg, K, Na in comparative abundance, lutein, and β-carotene as the major carotenoids with variations in the presented values due to genetic diversity, agricultural practices, harvesting stage, tissue as well as determination method (Wills & Rangga, 1996; Singh, Upadhyay, Prasad, Bahadur, & Rai, 2007; Watanabe, Musumi, & Ayugase, 2011; Kim et al., 2014; Bhandari, Jo, & Lee, 2015). Phytochemical compounds like indole compounds, b-sitosterol, benzyl isothiocyanate, and thiocyanate are actively found in kimchi, which possess various medicinal potentials such as antiobesity, anticancer, antioxidant, and antiatherosclerotic properties (Park & Rhee, 2005; Ahn, 2007; Park et al., 2014). The polyphenolic and glucosinolate contents of kimchi have been investigated due to its protective effects against various types of cancer, cardiovascular, and neurodegenerative diseases (Cohen, Kristal, & Stanford, 2000; Scalbert, Manach, Morand, Rémésy, & Jiménez, 2005; Han, Hu, & Lee, 2011; Rodríguez-Mateos et al., 2014). Sinapic acid has been reported as the major phenolic compound, whereas gluconapin, progoitrin, and glucobrassicin have been reported as the major glucosinolate (Hong, Kim, & Kim, 2011; Lee et al., 2014; Seong, Hwang, & Chung, 2016).

Radish and onion present in kimchi are very important sources of dietary phytochemicals such as polyphenols, thiosulfates, and anthocyanins (Slimestad, Fossen, & Vagen, 2007; Tedesco, Carbone, Spagnuolo, Minasi, & Russo, 2015). Onion is a major source of flavonoids, particularly quercetin glucosides (Srinivasan, 2014) with antiinflammatory property (Yang et al., 2015) and anticarcinogenic (Djuric, Severson, & Kato, 2012) action. Capsaicin and piperine are the major ingredients of red and black pepper, both having antioxidant activity (Arcaro et al., 2014). Garlic is rich in organosulfur compounds known for their antiinflammatory (Kumar et al., 2013) and potential cardioprotective activity (Stabler, Tejani, Huynh, & Fowkes, 2012; Ried, Toben, & Fakler, 2013). Ginger, another major ingredient, has a wide range of biological activities, including anticancer (Brahmbhatt, Gundala, Asif, Shamsi, & Aneja, 2013) and antioxidant ones (Stoilova, Krastanov, Stoyanova, Denev, & Gargovaa, 2007).

3.5.3.4 Antimutagenic and anticancer effect

The antimutagenic effect of kimchi has been widely exhibited in in vivo and in vitro tests. The antimutagenic effect of kimchi against aflatoxin BI and *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG) has been proved in vitro in the Ames test and in the SOS chromates. Especially, optimally ripened kimchi showed the highest antimutagenicity compared with fresh and overripened kimchi. The kimchi extract also showed antimutagenic activity in the Drosophila wing test in vivo (Hwang et al., 1997).

Anticlastogenic activity of kimchi has been revealed in MMC-induced mice using the in vivo supravital staining micronucleus assay. Mouse embryonic C3H/10T1/2 cells usually form foci in culture media when exposed to a carcinogen. The foci that developed as types II and III have been correlated well with tumor formations of 50% and 85% in C3H mice, respectively. The transformation of C3H/10T1/2 cells decreased when kimchi extract (methanol soluble fraction, MSF) was added to the test system. When 200 lg MSF from 3-week-fermented (at 5°C) kimchi was added along with 3-methylcholanthrene (MCA) to the cells, then the number of types II and III foci formed was considerably reduced. The anticancer activity of kimchi has been shown in the in vitro assays on human cancer cell lines. Kimchi extracts inhibited the survival or growth of human cancer cells (AGS gastric cancer, HT-29 colon cancer, MG-63 osteosarcoma, HL-60 leukemia, and Hep 3B liver cancer) in the sulforhodamine B (SRB) assay, the MTT assay, and the growth inhibition test by inhibiting 3H thymidine incorporation in the cancer cells (Choi, Kim, Kim, & Park, 1997; Hur, Kim, & Park, 1999).

The kimchi dichloromethane fraction showed two bioactive compounds, β-sitosterol and a linoleic acid derivative, that arrested the tumor cells at the G2/M phase in the cell cycle and induced apoptosis of HL-60 human leukemia cells. HIRc-B cells were treated with dichloromethane fraction from kimchi and β-sitosterol that was identified as an active kimchi compound, followed by microinjection of oncogenic H-Rasv. The DNA synthesis of the cells was decreased, indicating that active compounds of kimchi affected the signal transduction pathway via Ras to the nucleus (Park et al., 2003). The anticancer effect of kimchi is mostly due to the regulation of apoptosis of cancer cells and the inhibition of inflammation. Optimum ripening of kimchi significantly induced apoptosis (expression of Bcl-2 family) of HT-29 human colon cancer cells. It upregulated the expression of Bax protein and Bax mRNA and showed downregulation of the Bcl-2 protein and Bcl-2 mRNA. The antiinflammatory and anticancer properties of kimchi were also verified using azoxymethane (AOM)- and dextran sulfate sodium (DSS)-induced colitis-associated colon cancer in mice (Kim, 2013).

3.5.3.5 Antioxidant and antiaging potential

Kimchi has been shown to contain antioxidative compounds that remove free radicals formed in the body by acting as hydrogen donors. The dichloromethane fraction of kimchi showed the highest antioxidant effect against LDL oxidation

in liver homogenates of an experimental group of rats fed the dichloromethane fraction. It inhibits thiobarbituric acid (TBARS) production in the presence of Cu^{2+}. The activity levels of other antioxidant enzymes like catalase, glutathione peroxidase (GSH-Px), Cu, Zn-superoxide (Cu, Zn-SOD), and Mn-superoxide (MnSOD) in the rats with kimchi solvent fractions added to the diet were lower than those of the control group that might be due to the lower rate of lipid oxidation observed in these groups (Kim, Kwon, & Song, 2000). The effect of kimchi intake on antiaging, free radical production, and antioxidative enzyme activities in the brains of senescence-accelerated mice was also evaluated (Kim, Ryu, Lee, & Park, 2002). Kimchi consumption restrained the increase in free radical production due to aging. Human keratinocyte cells (A431 human epidermoid carcinoma) and fibroblast (CCD-986SK human normal control) were cultured under oxidative stress provoked by paraquat, a superoxide anion generator, and hydrogen peroxide in the absence and presence of kimchi extract. The optimally ripened kimchi extract remarkably decreased the cytotoxicity induced by H_2O_2 in the keratinocyte cells. Fermented kimchi showed protective effects against the oxidative stress induced by these agents (Ryu et al., 1997).

3.5.3.6 Antiobesity effect

Kimchi has been proven to have the impact of dietary fiber and lower body weight by lowering the lipid profile. In a study done on HFD-fed SD rats, adding kimchi LAB powder (KL) resulted in lower body weight and exhibited lipid-lowering effects. Groups supplemented with 10% and 20% of KL had 13% and 15% lower body weights compared with those given HFD alone. Plasma triglyceride, cholesterol, and LDL levels were also significantly lower, and triglyceride and cholesterol were excreted in the feces by the KL group. *W. koreensis* OK1-6 cells isolated from kimchi showed antiobesity activity in differentiating 3T3-L1 cells. Both the spent culture medium extract and cytoplasmic fraction of *W. koreensis* OK1-6 cells extensively dwindled the triglyceride concentration and intracellular lipid accumulation in the treated groups, as compared with the control (Kwon, Cheigh, & Song, 2004).

3.5.3.7 Antimicrobial potential of LAB found in kimchi

LAB found in kimchi and all other fermented vegetable foods produce organic acids, including lactic acid, during vegetable fermentation. They also produce CO_2, ethanol, diacetyl, H_2O_2, and bacteriocins. Because of these compounds, these functional foods have a profound antimicrobial effect, especially antibacterial and antifungal effect on the system. *Le. mesenteroides*, *Leuconostoc carnosum*, *Lactobacillus curvatus*, *Lactobacillus pentosus*, *Weissella kimchii*, *W. cibaria* that were isolated and identified from kimchi showed variable antimicrobial activities against *B. subtilis*, *E. coli*, *Salmonella enteritidis*, *Salmonella paratyphi*, *S. typhi*, *S. aureus*, *Shigella boydii*, and *S. sonnei* depending on the strains. Five LAB strains from kimchi, *L. curvatus*, *Lactob. lactis*, *L. casei*, *L. pentosus*, and *L. sakei*, showed a wide range of antifungal activity against *Aspergillus flavus*, *Fusarium moniliforme*, *Penicillium commune*, and *Rhizopus oryzae. L. casei* and *L. pentosus* are active against the indicator fungus *A. fumigates* (Kim, 2005).

Kimchi LAB were also effective against carcinogenic and gastric ulcer causing bacteria *H. pylori*. The culture solutions of *S. thermophilus* and *Le. mesenteroides* from kimchi all inhibited *H. pylori*, which did not grow during the coculture with these strains. *H. pylori* was unable to colonize the stomach of *Lactobacillus salivarius*–infected gnotobiotic Balb/c mice; however, it could be colonized well, and cause gastritis, in germ-free mice. *L. salivarius* administered after *H. pylori* implantation could also eliminate colonization by the bacteria (Lee et al., 2010; Kabir et al., 1997).

3.5.3.8 Risks of kimchi consumption

The only potential risk of kimchi consumption that is known so far is its high salt concentration. The average salt content of baechu kimchi is 2.0%–5.0% and contemporary commercial kimchi contains 2.0%–2.5% of salt. Most fermented foods in Korea use salt (natural sea salt, NS) as a preservative and to support LAB fermentation. This could be reduced by the use of various kinds of salt for improving the functionality of kimchi. Purified salt, NS, natural sea salt without bittern (NS-B) or baked salt, and Gown salt (G) have all been used for preparing kimchi. Using the baked Gown salt or bamboo salt is also better for improving the quality and anticancer efficacy of kimchi. It is also proved that fermentation reduces the negative effects of salt.

Kimchi has various potential health benefits, and quality of kimchi can be increased by manipulating amounts of ingredients and fermentation conditions. Kimchi and kimchi LAB have antimutagenic and anticancer effects, antioxidative and antiaging effects, antiobesity, antiatherosclerotic, and immunity-potentiating functions, even though these have not undergone many RCTs using human subjects. Cruciferous vegetables used in kimchi improve the taste, preservation properties, and functionality of kimchi. Thus optimally prepared kimchi is one of the best functional foods.

3.5.4 Sauerkraut

Sauerkraut is one of the most widespread, fermented vegetable foods, made of preserved cabbage. It originated in the 4th century BCE and has spread worldwide. Sauerkraut is eaten frequently in Germany, but also in other European and Asian countries and the United States (Raak, Ostermann, Boehm, & Molsberger, 2014). Sauerkraut is a name given to the end product of fermenting cabbage. One of the reasons sauerkraut and kimchi are considered as superfoods is the fermentation makes all of the nutrients of the food more bioavailable than in its raw form. This means that all the vitamins, mineral, and phytonutrients that cabbage offers could become more bioavailable after fermentation (Orgeron, Corbin, & Scott, 2016).

3.5.4.1 Preparation of sauerkraut

The process of preparing industrial or homemade sauerkraut still relies on traditional methods. After washing and boiling, Chinese cabbages are soaked in a ceramic jar with salt, pressed under a stone, and finally fermented at low temperature (below 15°C) for more than 1 month (Varzakas, Zakynthinos, Proestos, & Radwanska, 2017). The cabbage is soaked in 2.3%–3.0% salt, which is left to undergo spontaneous fermentation, generally involving *Leuconostoc*, *Lactobacillus*, and *Pediococcus* species. The final product results in a preserved cabbage with very low pH (Orgeron et al., 2016).

Fermentation is initiated by heterofermentative LAB (*Leuconostoc*) before shifting to a homofermentative stage between days 3 and 7 (Varzakas et al., 2017), and the process is finally completed by homofermentative LAB (*Lactobacillus*). As reported in a previous study, *Le. mesenteroides* and *L. plantarum* were identified as the major species at the initial and mid-late stage of sauerkraut fermentation, respectively. These bacteria are all Gram-positive, nonsporulating, nongelatin liquifying, and microaerophilic species. *Le. mesenteroides* can ferment glucose into approximately 45% lactic acid, 25% carbon dioxide, and 25% acetic acid and ethanol (Yang et al., 2019).

3.5.4.2 The nutritional value of sauerkraut

Sauerkraut (homemade and commercial) has been shown to contain many LAB species of bacteria like *Bifidobacterium dentium*, *E. faecalis*, *L. casei*, *L. delbrueckii*, *Staphylococcus epidermidis*, *L. sakei*, *L. curvatus*, *L. plantarum*, *L. brevis*, *W. confusa*, *Lactoc. lactis*, and *Enterobacteriaceae*, by culture techniques (Bati & Boyko, 2016). Adding a starter culture of *L. casei*, 11MZ-5-1 produced a sauerkraut containing predominantly *Lactobacillus* and *Lactococcus*, compared to spontaneous sauerkraut which, along with *Lactococcus* and *Lactobacillus*, also contained *Enterobacter* and *Pseudomonas* and had a more variable microbial composition. *L. paracasei*, commonly found in sauerkraut, produces a broad-spectrum bacteriocin that may play a role in sauerkraut preservation (Ge et al., 2009).

3.5.4.3 Therapeutic potential of sauerkraut

3.5.4.3.1 Detoxification Oral administration of sauerkraut juice in Wistar rats showed increased activity of glutathione *S*-transferase (GST) and NAD(P)H quinone oxidoreductase 1 (NQO1) enzymes. These are key players in liver and kidney detoxification (Krajka-Kuzniak, Szaefer, Bartoszek, & Baer-Dubowska, 2011).

3.5.4.3.2 Anticancer and antioxidant potential Certain LAB present in sauerkraut generate CLA, which show anticarcinogenic and antiatherosclerotic activity in animals. *L. plantarum* P2 isolated from sauerkraut significantly induced TNF-α and IL-12 expression and prevented adhesion and invasion of Caco-2 cells by *S. enteritidis* (Feng, Liu, Yang, & Zhao, 2015). Glucosinolate breaks down products, including kaempferol that is a flavonoid compound isothiocyanates, indole-3-carbinol, goitrin, allyl cyanide, and nitriles. Kaempferol has been shown to have radical scavenging activity, to protect from oxidative damage and to attenuate cytokine-induced ROS in vitro (Devi et al., 2015).

3.5.4.3.3 Antimicrobial effect and gut health Isothiocyanates present in sauerkraut have been shown to have antimicrobial properties, preventing the growth of many bacteria, including *E. coli*, *Clostridium difficile*, *Clostridium jejuni,* and *Clostridium perfringens*. This property of sauerkraut has led to clinical trials against functional bowel disorders. A randomized double-blind trial compared the effects of sauerkraut containing viable LAB on gastrointestinal symptoms and microbiota in 58 patients with irritable bowel syndrome (IBS) (Nielsen et al., 2018). Patients were randomly asked to consume 75 g/day pasteurized (control) or unpasteurized (intervention) sauerkraut containing LAB for 6 weeks. There was a significant reduction in IBS severity scoring system score between baseline and end of trial in both study groups; however, there was no difference in symptoms between the diet groups; 16S rRNA sequencing revealed no difference in microbiota composition between the groups. This suggests that the perceived health benefit of sauerkraut is independent of the live microbes.

3.5.4.4 Risks of sauerkraut consumption

Another study in Chinese participants suggests that taking larger amount of sauerkraut may be associated with gastrointestinal cancers. This case–control study found that the highest sauerkraut intake was associated with a greater risk of laryngeal cancer compared to the lowest [odds ratio (OR) 7.27]. This may be to the high salt content of sauerkraut (Zheng et al., 1992). But the high potassium content of sauerkraut acts to counter the hypertensive effects of added salt. The possibility of reducing the salt requirement for cabbage fermentation depends upon the addition of an appropriate starter culture. Studies have proved that fermenting with 50% less salt results in increased variability among many quality factors, including unpredictable softening and generation of off-flavors. Addition of *Lactobacillus mesenteroides* starter culture provided the appropriate fermentation regardless of salt level, ensuring the production of high-quality sauerkraut (Yang et al., 2019).

These study outcomes are promising in terms of positive effects of sauerkraut in humans, for example, as a vehicle of probiotics, in cancer prevention, its free radical scavenging and antiinflammatory potential. However, evidence for these implications is low (Gille, Schmid, Walther, & Vergères, 2018). Further studies are required to elucidate the mechanisms of this effect. There is little evidence for effects of sauerkraut on other health conditions. However, it is considered to be a probiotic superfood with many beneficial effects on gut health.

4 Conclusion

The various mechanisms underlying the beneficial effects of fermented foods are gaining more attention, and results of in vitro and in vivo animal trials have proven that there is a large scope for the use of probiotic foods for therapeutic purposes. The accumulating results from clinical and animal studies have brought to light the effects of these functional foods as having immense effects on gut health and gut microbiota. Fermented foods are gaining popularity among consumers for their possible therapeutic and high market value. As more studies are published in the future, it would be helpful in confirming the beneficial effects of fermented functional foods.

References

Adolfsson, O., Meydani, S. N., & Russell, R. M. (2004). Yogurt and gut function. *The American Journal of Clinical Nutrition*, *80*, 245–256. 1,2 Review Article, Printed in USA. American Society for Clinical Nutrition.

Ahn, S. J. (2007). *The effect of kimchi powder supplement on the body weight reduction of obese adult women*. Busan: Pusan National University. MS thesis.

Ahola, A. J., Knnuttila-Yli, H., Suomalainen, T., Poussa, T., Ahlsrom, A., & Meurman, J. H. (2002). Short-term consumption of probiotic containing cheese and its effect on dental caries risk factors. *Archives of Oral Biology*, *47*(11), 799–804.

Aiba, Y., Suzuki, N., Kabir, A. M. A., Takagi, A., & Koga, Y. (1998). Lactic acid-mediated suppression of *Helicobacter pylori* by the oral administration of *Lactobacillus salivarius* as a probiotic in a gnotobiotic murine model. *The American Journal of Gastroenterology*, *93*, 2097–2101.

Alm, L. (1982). Effect of fermentation on lactose, glucose and galactose content in milk and suitability of fermented milk products for lactose-deficient individuals. *Journal of Dairy Science*, *65*, 346–352.

Aneja, R. P., & Murthi, T. N. (1990). Conjugated linoleic acid contents of Indian curd and ghee. *Indian Journal of Dairy Science*, *43*, 231–238.

Arcaro, C. A., Gutierres, V. O., Assis, R. P., Moreira, T. F., Costa, P. I., & Baviera, A. (2014). Piperine, a natural bioenhancer, nullifies the antidiabetic and antioxidant activities of curcumin in streptozotocin-diabetic rats. *PLoS One*, *9*, e113993. doi: 10.1371/journal.pone.0113993.

Ash, A., & Wilbey, A. (2010). The nutritional significance of cheese in the UK diet. *International Journal of Dairy Technology*, *63*, 305–319.

Ayebo, A. D., Shahani, K. M., & Dam, R. (1981). Antitumor component(s) of yogurt: Fractionation. *Journal of Dairy Science*, *64*(99), 2318–2323.

Barnes, S., Coward, L., Kirk, M., & Sfakianos, J. (1998). HPLC–mass spectrometry analysis of isoflavones. *Proceedings of the Society Experimental Biology and Medicine*, *217*, 254–262.

Baschali, A., Tsakalidou, E., Kyriacou, A., Karavasiloglou, N., & Matalas, A. L. (2017). Traditional low-alcoholic and non-alcoholic fermented beverages consumed in European countries: A neglected food group. *Nutrition Research Reviews*, *30*(1), 1–24.

Bati, V. V., & Boyko, N. V. (2016). The microbial diversity and its dynamics in the ethnic fermented foods of the black sea region. *Microbiological Journal*, *78*, 53–64.

Bernet, M. F., Brassart, D., Neeser, J. R., & Servin, A. L. (1994). *Lactobacillus acidophilus* LA 1 binds to cultured human intestinal cell lines and inhibits cell attachment and cell invasion by enterovirulent bacteria. *Gut*, *35*, 483–489.

Bernet, M. F., Brassart, D., Neeser, J. R., & Servin, A. L. (1993). Adhesion of human bifidobacterial strains to cultured human intestinal epithelial cells and inhibition of enteropathogen-cell interactions. *Applied and Environmental Microbiology*, *59*, 4121–4128.

Bhandari, S. R., Jo, J. S., & Lee, J. G. (2015). Comparison of glucosinolate profiles in different tissues of nine *Brassica* crops. *Molecules*, *20*, 15827–15841. doi: 10.3390/molecules200915827.

Blandino, A., Al-Asceri, M. E., Pandiella, S. S., Cantero, D., & Webb, C. (2003). Cereal based fermented foods and beverages. *Food Research International*, *36*, 527–543.

Block, G., & Abrams, B. (1993). Vitamin and mineral status of women of childbearing potential. *Annals of the New York Academy of Sciences*, *678*, 244–254.

Borruel, N., Carol, M., & Casellas, F. (2002). Increased mucosal tumour necrosis factor alpha production in Crohn's disease can be downregulated ex vivo by probiotic bacteria. *Gut*, *51*, 659–664.

Bourlioux, P., & Pochart, P. (1988). Nutritional and health properties of yogurt. *World Review of Nutrition and Dietetics*, *56*, 217–258.

Brahmbhatt, M., Gundala, S. R., Asif, G., Shamsi, S. A., & Aneja, R. (2013). Ginger phytochemicals exhibit synergy to inhibit prostate cancer cell proliferation. *Nutrition and Cancer*, *65*, 263–272. doi: 10.1080/01635581.2013.7 49925.

Breidt, F., McFeeters, R. F., Perez-Diaz, I., & Lee, C. -H. (2013). Chapter 33—Fermented vegetables. In M. P. Doyle, & R. L. Buchanan (Eds.), *Food microbiology: Fundamentals and frontiers* (4th ed.). Washington, DC: ASM Press.

Bronner, F., & Pansu, D. (1999). Nutritional aspects of calcium absorption. *The Journal of Nutrition*, *129*, 9–12.

Butikofer, U., Meyer, J., Sieber, R., & Wechsler, D. (2007). Quantification of the angiotensin-converting enzyme-inhibiting tripeptides Val-Pro-Pro and Ile-Pro-Pro in hard, semi-hard and soft cheeses. *International Dairy Journal*, *17*, 968–975.

Buttriss, J. (1997). Nutritional properties of fermented milk products. *International Journal of Dairy Technology*, *50*, 21–27.

Caldini, G., Trotta, F., Corsetti, A., & Cenci, G. (2008). Evidence for in vitro anti-genotoxicity of cheese non-starter lactobacilli. *Antonie Van Leeuwenhoek*, *93*, 51–59.

Chakraborty, R., & Roy, S. (2018). Exploration of the diversity and associated health benefits of traditional pickles from the Himalayan and adjacent hilly regions of Indian subcontinent. *Journal of Food Science and Technology*, *55*, 1599–1613.

Chaltow, L., & Trener, N. (2002). *Probiotics*. Natasha Trenev. Website www.Natren.com.

Chandan, R. C., & Shahani, K. M. (1993). Yogurt. In Y. H. Hui (Ed.), *Dairy science and technology handbook* (pp. 1–57). New York, NY: VCH Publishers, Inc.

Charalampopoulos, D., Pandiella, S. S., & Webb, C. (2002). Growth studies of potentially probiotic lactic acid bacteria in cereal-based substrates. *Journal of Applied Microbiology*, *92*, 851–859.

Cheigh, H. S., & Park, K. Y. (1994). Biochemical, microbiological, and nutritional aspects of kimchi (Korean fermented vegetable products. *Critical Reviews in Food Science and Nutrition*, *34*, 175–203.

Cho, J., Lee, D., Yang, C., Jeon, J., Kim, J., & Han, H. (2006). Microbial population dynamics of kimchi, a fermented cabbage product. *FEMS Microbiology Letters*, *257*, 262–267.

Choi, M. W., Kim, K. H., Kim, S. H., & Park, K. Y. (1997). Inhibitory effects of kimchi extracts on carcinogen-induced cytotoxicity and transformation in C3H10T1/2 cells. *Journal of Food Science and Nutrition*, *2*, 241–245.

Choi, I. H., Noh, J. S., Han, J., Kim, H. J., Han, E., & Song, Y. (2013). Kimchi: A fermented vegetable, improves serum lipid profiles in healthy young adults: Randomized clinical trial. *Journal of Medicinal Food*, *16*, 223–229.

Chun, J. K., Kim, K. M., & Woo, D. H. (1999). Automation of kimchi fermentation based on pattern analysis. *Food Engineering Program*, *3*, 181–185.

Codex Alimentarius Commission (Codex). (2001). Codex standard for kimchi. *Codex standard* (Vol. 223). Rome, Italy: Food and Agriculture Organization of the United Nations.

Cohen, J. H., Kristal, A. R., & Stanford, J. L. (2000). Fruit and vegetable intakes and prostate cancer risk. *Journal of the National Cancer Institute*, *92*, 61–68. doi: 10.1093/jnci/92.1.61.

Corsetti, A., Caldini, G., Mastrangelo, M., Trotta, F., Valmorri, S., & Cenci, G. (2008). Raw milk traditional Italian ewe cheeses as a source of *Lactobacillus casei* strains with acid-bile resistance and antigenotoxic properties. *International Journal of Food Microbiology*, *125*, 330–335.

Corthier, G. (2004). The health benefits of probiotics. Editorial, available from: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.198.1141 & Cited 01.07.13.

da Cruz, A. G., Buriti, F. C. A., de Souza, C. H. B., Faria, J. A. F., & Saad, S. M. I. (2009). Probiotic cheese: Health benefits, technological and stability aspects. *Review – Trends in Food Science & Technology*, *20*, 344–354.

Danciu, C., Antal, D. S., Ardelean, F., Rodica Chiş, A., Ŝica, C., Andrica, F., et al. (2017). New insights regarding the potential health benefits of isoflavones. *Flavonoids—From biosynthesis to human health*. doi: 10.5772/67896.

Davis, J., Iqbal, M. J., Steinle, J., Oitker, J., Higginbotham, D. A., & Peterson, R. G. (2007). Soy protein influences the development of the metabolic syndrome in male obese ZDFxSHHF rats. *Hormone and Metabolic Research*, *37*, 316–325.

De Simone, C., Salvadori, B. B., Negri, M., Ferrazzi, M., Baldinelli, L., & Vesely, R. (1986). The adjuvant effect of yogurt on production of gamma-interferon by Con A stimulated human peripheral blood lymphocytes. *Nutrition Reports International*, *33*, 419–433.

Deeth, H. C., & Tamime, A. Y. (1981). Yogurt, nutritive and therapeutic aspects. *Journal of Food Protection*, *44*, 78–86.

Demir, N., Bahceci, K. S., & Acar, J. (2006). The effects of different initial *Lactobacillus plantarum* concentrations on some properties of fermented carrot juice. *Journal of Food Processing and Preservation*, *30*, 352–363. doi: 10.1111/j.1745-4549.2006.00070.x.

Devi, K. P., Malar, D. S., Nabavi, S. F., Sureda, A., Xiao, J., Nabavi, S. M., et al. (2015). Kaempferol and inflammation: From chemistry to medicine. *Pharmacological Research*, *99*, 1–10.

Dharaneedharan, S., & Heo, M. S. (2016). Korean traditional fermented foods—A potential resource of beneficial microorganisms and their applications. *Journal of Life Sciences*, *26*, 496–502. doi: 10.5352/JLS.2016.26.4.496.

Di Cagno, R., Coda, R., Angelis, M. D., & Gobbetti, M. (2013). Exploitation of vegetables and fruits through lactic acid fermentation. *Food Microbiology*, *33*, 1–10. doi: 10.1016/j.fm.2012.09.003.

Dimidi, E., Rose Cox, S., Rossi, M., & Whelan, K. (2019). Fermented foods: Definitions and characteristics, impact on the Gut microbiota and effects on gastrointestinal health and disease. *Nutrients*, *11*(8), 1806. doi: 10.3390/nu11081806.

Djuric, Z., Severson, R. K., & Kato, I. (2012). Association of dietary quercetin with reduced risk of proximal colon cancer. *Nutrition and Cancer*, *64*, 351–360. doi: 10.1080/01635581.2012.658950.

Dufresne, C., & Farnworth, E. (2000). Tea, kombucha, and health: A review. *Food Research International*, *33*(6), 409–421.

Dupont, D., Arnould, C., Rolet-Repecaud, O., Duboz, G., Faurie, F., Martin, B., et al. (2006). Determination of bovine lactoferrin concentrations in cheese with specific monoclonal antibodies. *International Dairy Journal*, *16*, 1081–1087.

El-Abbadi, N. H., Dao, M. C., & Meydani, S. N. (2014). Yogurt: Role in healthy and active aging. *The American Journal of Clinical Nutrition*, *99*(Suppl.), 1263S–1270S.

Elbarbary, H. A., Abdou, A. M., Park, E. Y., Nakamura, Y., & Mohamed, H. A. (2010). Novel antibacterial lactoferrin peptides generated by rennet digestion and autofocusing technique. *International Dairy Journal*, *20*, 646–651.

Erickson, David R. (1995). *Practical handbook of soybean processing and utilization*. AOCS Press. 978-0-935315-63-9.

Etchells, J. L., Bell, T. A., & Moore, W. R., Jr. (1976). Refrigerated dill pickles, questions and answers. *Pickle Pak Science*, *5*, 1–20.

Everett, D. W., & Auty, M. A. E. (2008). Cheese structure and current methods of analysis. *International Dairy Journal*, *18*(7), 759–773.

FAO (2003). *Codex standard for fermented milks*. Rome, Italy: Codex Alimentarius Commission.

Farke, N. Y. (2004). Cheese technology. *International Journal of Dairy Technology*, *57*(1), 91–98.

Favier, J. C., & Dorsainvil, E. (1987). Composition des fromages de chèvre. *Cahiers de Nutrition et de Diététique*, 17–123.

Feng, J., Liu, P., Yang, X., & Zhao, X. (2015). Screening of immunomodulatory and adhesive *Lactobacillus* with antagonistic activities against *Salmonella* from fermented vegetables. *World Journal of Microbiology and Biotechnology*, *31*, 1947–1954.

Fernandez, A. G., Garcia, P. G., & Balbuena, M. B. (1995). Olive fermentations. In H. -J. Rehm, & G. Reed (Eds.), *Biotechnology* (pp. 593–627). New York, NY: VCH Publishers.

Ferrazzano, G. F., Cantile, T., Quarto, M., Ingenito, A., Chianese, L., & Addeo, F. (2008). Protective effect of yogurt extract on dental enamel demineralization in vitro. *Australian Dental Journal*, *53*, 314–319.

Ferrières, J. (2004). The French paradox: Lessons for other countries. *Heart*, *90*, 107–111.

Fleming, H. P., Kyung, K. -H., & Breidt, F., Jr. (1995). Vegetable fermentations. In H. -J. Rehm, & G. Reed (Eds.), *Biotechnology* (pp. 629–661). New York, NY: VCH Publishers.

Fox, P. F., Guinee, T. P., Cogan, T. P., & McSweeney, P. L. H. (2000). Fundamentals of cheese science. *Gaithersburg: Aspen*, 1–9.

Gaggia, F., Baffoni, L., Galiano, M., Nielson, S. D., Jakobsen, R., Castro-Mejia, L. J., et al. (2018). Kombucha beverage from green, black and rooibos teas: A comparative study looking at microbiology, chemistry and antioxidant activity. *Nutrients*.

Gardiner, G., Stanton, C., Lynch, P. B., Collins, J. K., Fitzgerald, G., & Ross, R. P. (1999). Evaluation of cheddar cheese as a food carrier for delivery of a probiotic strain to the gastrointestinal tract. *Journal of Dairy Science*, *82*(7), 1379–1387.

Ge, J., Ping, W., Song, G., Du, C., Ling, H., Sun, X., et al. (2009). Paracin 1.7, a bacteriocin produced by *Lactobacillus paracasei* HD1.7 isolated from Chinese cabbage sauerkraut, a traditional Chinese fermented vegetable food. *Acta Microbiologica Sinica*, *49*, 609–616.

Gille, D., Schmid, A., Walther, B., & Vergères, G. (2018). Fermented food and non-communicable chronic diseases: A review. *MDPI Nutrients*, *10*(4), 448. doi: 10.3390/nu10040448.

Gitanjali, B. S., & Manda, S. (2016). Fermented products of India and its implication: A review. *Asian Journal of Dairy and Food Research*, *35*, 1–9.

Glass, R. I., Lew, J. F., Gangarosa, R. E., LeBaron, C. W., & Ho, M. S. (1991). Estimates of morbidity and mortality rates for diarrheal diseases in American children. *The Journal of Pediatrics*, *118*, S27–S33.

Gomez-Ruiz, J. A., Ramos, M., & Recio, I. (2002). Angiotensin converting enzyme inhibitory peptides in Manchego cheeses manufactured with different starter cultures. *International Dairy Journal*, *12*, 697–706.

Goodenough, E. R., & Kleyn, D. H. (1976). Influence of viable yogurt microflora on digestion of lactose by the rat. *Journal of Dairy Science*, *59*, 601–606.

Gottschalk, G. (1986). *Bacterial metabolism* (2nd ed.). New York, NY: Springer-Verlag. pp. 208-220.

Greenwalt, C. J., Steinkraus, K. H., & Ledford, R. A. (2000). Kombucha, the fermented tea: Microbiology, composition, and claimed health effects. *Journal of Food Protection*, *63*(7), 976–981.

Gross, B. L., & Zhao, Z. (2014). Archaeological and genetic insights into the origins of domesticated rice. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, 6190–6197.

Grundelius, A. U., Lodaite, K., Östergren, K., Paulsson, M., & Dejmek, P. (2000). Syneresis of submerged single curd grains and curd rheology. *International Dairy Journal*, *10*(7), 489–496.

Gupta, A., & Tiwari, S. K. (2014). Probiotic potential of *Lactobacillus plantarum* LD1 isolated from batter of dosa, a south Indian fermented food. *Probiotics Antimicrobial Proteins*, *6*, 73–81.

Halpern, G. M., Vruwink, K. G., Van de Water, J., Keen, C. L., & Gershwin, M. E. (1991). Influence of long-term yogurt consumption in young adults. *International Journal of Immunotherapy*, *7*, 205–210.

Han, H. U., Lim, C. R., & Park, H. K. (1990). Determination of microbial community as an indicator of Kimchi fermentation. *Korean Journal of Food Science and Technology*, *22*, 26–32.

Han, W., Hu, W., & Lee, Y. M. (2011). Anti-cancer activity of human colon cancer (HT-29) cell line from different fraction of Zanthoxylum schnifolium fruits. *Korean Journal of Pharmacognosy*, *42*, 282–287.

Hatakka, K., Ahola, A. J., Yli-Knuuttila, H., Richardson, M., Poussa, T., & Meurman, J. H. (2007). Probiotics reduce the prevalence of oral Candida in the elderly e a randomized controlled trial. *Journal of Dental Research*, *86*(2), 125–130.

Hiremath, U. S., Vaidehi, M. P., & Mushtari, B. J. (2002). Effect of fermented tea on the blood sugar levels of NIDDM subjects. *The Indian Practitioner*, *55*, 423–425.

Hong, E., Kim, S. J., & Kim, G. H. (2011). Identification and quantitative determination of glucosinolates in seeds and edible parts of Korean Chinese cabbage. *Food Chemistry*, *128*, 1115–1120. doi: 10.1016/j.foodchem.2010.11.102.

Hsieh, M. L., & Chou, C. C. (2006). Mutagenicity and antimutagenic effect of soymilk fermented with lactic acid bacteria and bifidobacteria. *International Journal of Food Microbiology*, *111*, 43–47.

Hur, Y. M., Kim, S. H., & Park, K. Y. (1999). Inhibitory effects of kimchi extracts on the growth and DNA synthesis of human cancer cells. *Journal of Food Science and Nutrition*, *4*, 107–112.

Hwang, S. Y., Hur, Y. M., Choi, Y. H., Rhee, S. H., Park, K. Y., & Lee, W. H. (1997). Inhibitory effect of kimchi extracts on mutagenesis of aflatoxin B1. *Environmental Mutagens and Carcinogens*, *17*, 133–137.

International Dairy Federation. (2007). The world dairy situation 2007. *Bulletin—International Dairy Federation*, *423*, 5–18.

Iyer, B. K., Singhal, R. S., & Ananthanarayan, L. (2013). Characterization and in vitro probiotic evaluation of lactic acid bacteria isolated from idli batter. *Food Science and Technology*, *50*, 1114–1121.

Jayabalan, R., Malbasa, R. V., Loncar, E. S., Vitas, J. S., & Sathishkumar, M. (2014). A review on kombucha tea-microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. *Comprehensive Reviews in Food Science and Food Safety*, *13*(4), 538–550.

Jayabalan, R., Malini, K., Sathishkumar, M., Swaminathan, K., & Yun, S. E. (2010). Biochemical characteristics of tea fungus produced during Kombucha fermentation. *Food Science and Biotechnology*, *19*, 843–847.

Jayabalan, R., Subathradevi, P., Marimuthu, S., Sathishkumar, M., & Swaminathan, K. (2008). Changes in free radical scavenging ability of kombucha tea during fermentation. *Food Chemistry*, *109*, 227–234.

Jeong, J. -K., Kim, Y. -W., Choi, H. -S., Lee, D. S., Kang, S. -A., & Park, K. -Y. (2011). Increased quality and functionality of kimchi when fermented in Korean earthenware (onggi). *International Journal of Food Science and Technology*, *46*, 2015–2021.

Kabir, A. M. A., Aiba, Y., Takagi, A., Kamiya, S., Miwa, T., & Koga, T. (1997). Prevention of *Helicobacter pylori* infection by lactobacilli in a gnotobiotic murine model. *Gut*, *41*, 49–55.

Kaczmarczyk, D., & Lochynski, S. (2014). Products of biotransformation of tea infusion—Properties and application. *Polish Journal of Natural Sciences*, *29*(4), 381–392.

Kapoor, A., Kanwar, P., & Gupta, R. (2010). Traditional recipes of districts Kangra of Himachal Pradesh. *Indian Journal of Traditional Knowledge*, *9*(2010), 282–288.

Kaup, S. M., Shahani, K. M., Amer, M. A., & Peo, E. R. (1987). Bioavailability of calcium in yogurt. *Milchwissenschaft*, *42*, 513–516. (in German).

Kim, H. J., Kwon, M. J., & Song, Y. O. (2000). Effects of solvent fractions of Korean cabbage kimchi on antioxidative enzyme activities and fatty acid composition of phospholipid of rabbit-fed 1% cholesterol diet. *Korean Society of Food Science and Nutrition*, *29*, 900–907.

Kim, H. Y. (2013). *Fermentation properties and inhibitory effects of anticancer functional baechu kimchi on AOM/DSS induced colitis-associated colon cancer in Balb/c mice*. Busan, Korea: Pusan National University. MS thesis.

Kim, J. D. (2005). Antifungal activity of lactic acid bacteria isolated form kimchi against *Aspergillus fumigates*. *Mycobiology*, *33*, 210–214.

Kim, J. H., Ryu, J. D., Lee, H. G., & Park, J. H. (2002). The effect of kimchi on production of free radicals and antioxidative enzyme activities in the brain of SAM. *Journal of the Korean Society of Food Science and Nutrition*, *31*, 117–123.

Kim, J. J., Maria-John, K. M., Moon, H. K., Jin, K., Enkhtaivan, G., & Kim, D. H. (2014). Morphological and biochemical variation of Chinese cabbage (*Brassica rapa* spp. *pekinensis*) cultivated using different agricultural practices. *Journal of Food Composition and Analysis*, *36*, 12–23. doi: 10.1016/j.jfca.2014.06.009.

Kim, M. J., & Chun, J. S. (2005). Bacterial community structure in kimchi, a Korean fermented vegetable food, as revealed by 16S rRNA gene analysis. *International Journal of Food Microbiology*, *103*, 91–96.

Kolars, J. C., Levitt, M. D., Aouji, M., & Savaiano, D. A. (1984). Yogurt-an autodigesting source of lactose. *The New England Journal of Medicine*, *310*, 1–3.

Kosikowski, F. V., & Mistry, V. V. (1997). Cheese and fermented milk foods. In F. V. Kosikowski (Ed.), *Origins and principles* (pp. 539). (Vol. 1). Westport, CT.

Kovacevic, Z., Davidovic, G., Vuckovic-Filipovic, J., Janicijevic-Petrovic, M., Janicijevic, K., & Popovic, A. (2014). A toxic hepatitis caused the kombucha tea—Case report. *Macedonian Journal of Medical Sciences*, *7*, 128–131.

Krajka-Kuzniak, V., Szaefer, H., Bartoszek, A., & Baer-Dubowska, W. (2011). Modulation of rat hepatic and kidney phase II enzymes by cabbage juices: Comparison with the effects of indole-3-carbinol and phenethyl isothiocyanate. *British Journal of Nutrition*, *105*, 816–826.

Kruif, C.G. & Holt, C. (2006). Casein micelle structure, functions and interactions. In P.F., Fox., P.L.H., McSweeney, (Eds.) Advanced Dairy Chemistry-Proteins.

Kuhlsen, N., Pfeuffer, M., Soustre, Y., MacGibbon, A., Lindmark-Mansson, H., & Schrezenmeir, J. (2005). Trans fatty acids: Scientific progress and labelling. *Bulletin of the International Dairy Federation*, *393*, 1–20.

Kumar, R., Chhatwal, S., Arora, S., Sharma, S., Singh, J., & Singh, N. (2013). Antihyperglycemic, antihyperlipidemic, anti-inflammatory and adenosine deaminase–lowering effects of garlic in patients with type 2 diabetes mellitus with obesity. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, *6*, 49–56. doi: 10.2147/DMSO.S38888.

Kunji, E. R. S., Mierau, I., Hagting, A., Poolman, B., & Konings, N. (1996). The proteolytic system of lactic acid bacteria. *Antonie van Leeuwenhoek*, *70*, 187–221.

Kwak, H. -S., Palanivel, G., & Youn-Ho, H. (2011). Chapter 14: Nutritional benefits in cheese. In D. F. Richard (Ed.), *Cheese: Types, nutrition and consumption* (pp. 269–289). Nova Science Publishers, Inc. 978-1-61209-828-9.

Kwon, J. Y., Cheigh, H. S., & Song, Y. O. (2004). Weight reduction and lipid lowering effects of kimchi lactic acid powder in rats fed high fat diets. *Korean Journal of Food Science and Technology*, *36*, 1014–1019.

Kwon, G. H., Lee, H. A., Park, J. Y., Kim, J. S., Lim, J. K., Park, C. S., et al. (2009). Development of a RAPD-PCK method for identification of *Bacillus* species isolated from Cheonggukjang. *International Journal of Food Microbiology*, *129*, 282–287.

Labonte, M. E., Couture, P., Richard, C., Desroches, S., & Lamarche, B. (2013). Impact of dairy products on biomarkers of inflammation: A systematic review of randomized controlled nutritional intervention studies in overweight and obese adults. *The American Journal of Clinical Nutrition*, *97*, 706–717.

Lal, G., Siddapp, G. S., & Tandon, G. L. (2010). *Chutneys, sauces and pickles, preservation of fruits and vegetables*. New Delhi: ICAR Publication. pp. 235-269.

Leal, J. M., Suarez, L. V., Jayabalan, R., Oros, J. H., & Escalante-Aburto, A. (2018). A review on health benefits of kombucha nutritional compounds and metabolites. *CyTA – Journal of Food*, *16*(1), 390–399.

Lee, Y. D., Yoo, H. L., Hwang, J. Y., Han, B. K., Choi, H. J., & Park, J. H. (2010). Antimicrobial effect of lactic acid bacteria isolated from kimchi and tarak on *Helicobacter pylori*. *The Korean Journal of Food And Nutrition*, *23*, 664–669.

Lee, C. H. (2001). *Fermentation technology in Korea*. Seoul, South Korea: Korea University Press.

Lee, C. H. (1994). Importance of lactic acid bacteria in non-dairy food fermentation. In C. H. Lee, J. Adler-Nissen, & G. Barwald (Eds.), *Lactic acid fermentation of non-dairy food and beverages* (pp. 8–25). Seoul, South Korea: Harnlimwon.

Lee, D., Kim, S., Cho, J., & Kim, J. (2008). Microbial population dynamics and temperature changes during fermentation of kimjang kimchi. *Journal of Microbiology*, *46*, 590–593.

Lee, G. I., Lee, H. M., & Lee, C. H. (2012). Food safety issues in industrialization of traditional Korean foods. *Food Control*, *24*, 1–5. doi: 10.1016/j.foodcont.2011.09.014.

Lee, J. H. (2009). Current studies on the community of lactic acid bacteria in kimchi a traditional Korean fermented food. *Milk Science*, *58*, 153–159.

Lee, M. K., Chun, J. H., Byeon, D. H., Chung, S. O., Park, S. U., & Park, S. (2014). Variation of glucosinolates in 62 varieties of Chinese cabbage (*Brassica rapa* L. spp. *pekinensis*) and their antioxidant activity. *LWT – Food Science and Technology*, *58*, 93–101. doi: 10.1016/j.lwt.2014.03.001.

Lee, S. W. (1975). Studies on the movements and inter-changes of vegetable pickles in China, Korea and Japan. *Journal of the Korean Society of Food Science and Nutrition*, *4*, 71–76.

Leroy, F., & de Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science and Technology*, *15*, 67–78.

Libudzisz, Z., & Piatkiewicz, A. (1990). Kefir production in Poland. *Dairy Industries International*, *55*, 31–33.

Lin, D. C. (2003). Probiotics as functional foods. *Nutrition in Clinical Practice*, *18*, 497–506.

Link-Amster, H., Rochat, F., Saudan, K. Y., Mignot, O., & Aeschlimann, J. M. (1994). Modulation of a specific humoral immune response and changes in intestinal flora mediated through fermented milk intakes. *FEMS Immunology and Medical Microbiology*, *10*, 55–64.

Lomholt, S. B., & Qvist, K. B. (1999). The formation of cheese curd. In B. A. Law (Ed.), *Technology of cheesemaking* (pp. 66–98). Boca Raton, FL: CRC Press.

Loones, A. (1989). Transformation of milk components during yogurt fermentation. In R. C. Chandan (Ed.), *Yogurt: Nutritional and health properties* (pp. 95–114). McLean, VA: National Yogurt Association.

Lucey, J. A., & Fox, P. F. (1993). Importance of calcium and phosphate in cheese manufacture: A review. *Journal of Dairy Science*, *76*, 1714–1724.

Maalouf, K., Baydoun, E., & Rizk, S. (2011). Kefr induces cell-cycle arrest and apoptosis in HTLV-1-negative malignant T-lymphocytes. *Cancer Management and Research*, *3*, 39.

Malbaša, R. V., Lončar, E. S., Vitas, J. S., & Čanadanović-Brunet, J. M. (2011). Influence of starter cultures on the antioxidant activity of kombucha beverage. *Food Chemistry*, *127*, 1727–1731.

Massi, M., Vitali, B., Federici, F., Matteuzzi, D., & Brigidi, P. (2004). Identification method based on PCR combined with automated ribotyping for tracking probiotic *Lactobacillus* strains colonizing the human gut and vagina. *Journal of Applied Microbiology*, *96*, 777–786.

McCabe, L. D., Martin, B. R., McCabe, G. P., Johnston, C., Weaver, C. M., & Peacock, M. (2004). Dairy intakes affect bone density in the elderly. *The American Journal of Clinical Nutrition*, *80*, 1066–1074.

McFeeters, R. F., Fleming, H. P., & Thompson, R. L. (1982). Malic acid as a source of carbon dioxide in cucumber fermentations. *Journal of Food Science*, *47*, 1862–1865.

Medici, M., Vinderola, C. G., & Perdigon, G. (2004). Gut mucosal immunomodulation by probiotic fresh cheese. *International Dairy Journal*, *14*(7), 611–618.

Metchnikoff, E. (1901). Sur la flore du corps humain (On the flora of the human body). *The Manchester Literary and Philosophical Society*, *45*, 1–38. (in French).

Mheen, T. -I., & Kwon, T. -W. (1984). Effect of temperature and salt concentration on kimchi fermentation. *Korean Journal of Food Science and Technology*, *16*, 443–450.

Mckinley, M. C. (2005). The nutrition and health benefits of yoghurt. *International Journal of Dairy Technology*, *58*(1).

Midolo, P. D., Lambert, J. R., Hull, R., Luo, F., & Grayson, M. L. (1995). In vitro inhibition of *Helicobacter pylori* NCTC 11637 by organic acids and lactic acid bacteria. *Journal of Applied Microbiology*, *79*, 475–479.

Miettinen, M., Vuopio-Varkila, J., & Varkila, K. (1996). Production of human tumor necrosis factor-alpha, interleukin-6 and interleukin-10 is induced by lactic acid bacteria. *Infection and Immunity*, *64*, 5403–5405.

Miranda, B., Lawton, N. M., Tachibana, S. R., Swartz, N. A., & Hall, W. P. (2016). Titration and HPLC characterization of Kombucha fermentation: A laboratory experiment in food analysis. *Journal of Chemical Education*, *93*(10), 1770–1775.

Mohan, V., Spiegelman, D., Sudha, V., Gayathri, R., Hong, B., Praseena, K., et al. (2014). Effect of brown rice, white rice, and brown rice with legumes on blood glucose and insulin responses in overweight Asian Indians: A randomized controlled trial. *Diabetes Technology & Therapeutics*, *16*, 317–325.

Moktan, B., Roy, A., & Sarkar, P. K. (2011). Antioxidant activities of cereal-legume mixed batters as influenced by process parameters during preparation of dhokla and idli, traditional steamed pancakes. *International Journal of Food Sciences and Nutrition*, *62*, 360–369.

Monika, S., Kumari, A., Kunzes, A., & Bhalla, T. C. (2016). Traditional pickles of Himachal Pradesh. *Indian Journal of Traditional Knowledge*, *15*(2), 330–336.

Mozaffarian, D., Hao, T., Rimm, E. B., Willett, W. C., & Hu, F. B. (2011). Changes in diet and lifestyle and long-term weight gain in women and men. *The New England Journal of Medicine.*, *364*, 2392–2404.

Mukherjee, K. P., Nema, N. K., Maity, N., & Sakar, B. K. (2013). Phytochemical and therapeutic potential of cucumber. *Fitoterapia*, 84227–84236. Review article.

Mukherjee, S. K., Albury, M. N., Pederson, C. S., Vanveen, A. G., & Steinkraus, K. H. (1965). Role of *Leuconostoc mesenteroides* in leavening the batter of idli, a fermented food of India. *Applied Microbiology*, *13*, 227–231.

Nagpal, R., Yadav, H., Puniya, A. K., Singh, K., Jain, S., & Marotta, F. (2007). Potential of probiotic and prebiotics for synbiotic functional dairy foods: An overview. *International Journal of Probiotics and Prebiotics*, *2*(2–3), 75–84.

Nguyen, C., & Carlin, F. (1994). The microbiology of minimally processed fresh fruits and vegetables. *Critical Reviews in Food Science and Nutrition*, *34*, 371–401.

Nguyen, N. K., Nguyen, P. B., Nguyen, H. T., & Le, P. H. (2015). Screening the optimal ratio of symbiosis between isolated yeast and acetic acid bacteria strain from traditional kombucha for high-level production of glucuronic acid. *LWT – Food Sciences Technological*, *64*, 1149–1155.

Nielsen, E. S., Garnås, E., Jensen, K. J., Hansen, L. H., Olsen, P. S., Ritz, C., et al. (2018). Lacto-fermented sauerkraut improves symptoms in IBS patients independent of product pasteurisation—A pilot study. *Food & Function*, *9*, 5323–5335.

Nurul, S. R., & Asmah, R. (2012). Evaluation of antioxidant properties in fresh and pickled papaya. *International Food Research Journal*, *19*, 1117–1124.

Ogilvy, S. (1977). *Making cheeses*. London: B.T. Batsford Ltd. 0713431687.

Orgeron, R., Corbin, A., & Scott, B. (2016). Sauerkraut: A probiotic superfood functional foods. *Health and Disease*, *6*(8), 536–543.

Oste, R., Jagerstad, M., & Andersson, I. (1997). Vitamins in milk and milk products. In P. F. Fox, & P. L. H. McSweeney (Eds.), *Advanced dairy chemistry – Volume 3: Lactose, water, salts and vitamins* (2nd ed., pp. 347–388). New York, NY: Kluwer Academic Plenum Publishers.

Otles, S., & Cagindi, O. (2003). Kefir: A probiotic dairy-composition, nutritional and therapeutic aspects. *Pakistan Journal of Nutrition*, *2*(2), 54–59.

Park, K. Y., Jeong, J. K., Lee, Y. E., & Daily, J. W. (2014). Health benefits of kimchi (Korean fermented vegetables) as a probiotic food. *Journal of Medicinal Food*, *17*, 6–20. doi: 10.1089/jmf.2013.3083.

Park, K. Y., & Rhee, S. H. (2005). Functional foods from fermented vegetable products: Kimchi (Korean fermented vegetables and functionality). In J. Shi, C. -T. Ho, & F. Shahidi (Eds.), *Asian functional foods* (pp. 341–380). Boca Raton, FL: CRC Press.

Park, K. Y., Cho, E. J., Rhee, S. H., Jung, K. O., Yi, S. J., & Jhun, B. H. (2003). Kimchi and an active component, beta-sitosterol, reduce oncogenic H-Rasv12-induced DNA synthesis. *Journal of Medicinal Food*, *6*, 151–156.

Park, K. Y., Kil, J. H., Jung, K. O., Kong, C. S., & Lee, L. M. (2006). Functional properties of kimchi (Korean fermented vegetables). *Acta Horticulturae*, *706*, 167–172. Avalaible online: http://www.actahort.org/books/706/706_19.htm Accessed 17.12.13.

Park, Y., & Pariza, M. W. (2007). Mechanisms of body fat modulation by conjugated linoleic acid (CLA). *Food Research International*, *40*, 311–323.

Parrot, S., Degraeve, P., Curia, C., & Martial-Gros, A. (2003). In vitro study on digestion of peptides in Emmental cheese: Analytical evaluation and influence on angiotensin I converting enzyme inhibitory peptides. *Nahrung*, *47*, 87–94.

Parvez, S., Malik, K. A., Ah Kang, S., & Kim, H. -Y. (2006). Probiotics and their fermented food products are beneficial for health. *Journal of Applied Microbiology*, *100*(6), 1171–1185.

Pedrosa, M. C., Golner, B. B., Goldin, B. R., Barakat, S., Dallal, G. E., & Russell, R. M. (1995). Survival of yogurt-containing organisms and *Lactobacillus gasseri* (ADH) and their effect on bacterial enzyme activity in the gastrointestinal tract of healthy and hypochlorhydric elderly subjects. *The American Journal of Clinical Nutrition*, *61*, 353–359.

Perdigon, G., Alvarez, S., Rachid, M., Aguero, G., & Gobbato, N. J. (1995). Immune system stimulation by probiotics. *Journal of Dairy Science*, *78*, 1597–1606.

Pointillart, A., Cayron, B., & Gueguen, L. (1986). Calcium and phosphorus utilization and bone mineralization in yogurt-fed pigs. *Sciences des Aliments*, *6*, 15–30.

Puri, P., Rattan, A., Bijlani, R. L., Mahapatra, S. C., & Nath, I. (1996). Splenic and intestinal lymphocyte proliferation response in mice fed milk or yogurt and challenged with *Salmonella typhimurium*. *International Journal of Food Sciences and Nutrition*, *47*, 391–398.

Purushothaman, D., Dhanapal, N., & Rangaswami, G. (1993). Indian idli, dosa, dhokla, khaman, and related fermentations. In K. H. Steinkraus (Ed.), *Handbook of indigenous fermented food*. New York, NY: Marcel Dekker.

Raak, C., Ostermann, T., Boehm, K., & Molsberger, F. (2014). Regular consumption of sauerkraut and its effect on human health: A bibliometric analysis. *Global Advances in Health and Medicine*, *3*, 12–18.

Radhakrishnamurty, R., Desikachar, H. S., Srinivasan, M., & Subrahmanyan, V. (1961). Studies on Idli fermentation. II. Relative participation of black gram flour and rice semolina in the fermentation. *Journal of Scientific and Industrial Research*, *20*, 342–345.

Rasic, J. L., & Kurmann, J. A. (1978). Yoghurt: Scientific grounds, technology, manufacture and preparations. In J. L. Rasic, & J. A. Kurmann (Eds.), *Fermented fresh milk products and their cultures* (Vol. 1). Copenhagen: Technical Dairy Publishing House.

Ray, M., Ghosh, K., Singh, S., & Mondal, K. C. (2016). Folk to functional: An explorative overview of rice-based fermented foods and beverages in India. *Journal of Ethnic foods*, *3*(1), 5–18. doi: 10.1016/j.jef.2016.02.002. Review.

Reddy, B. S., & Rivenson, A. (1993). Inhibitory effect of *Bifidobacterium longum* on colon, mammary, and liver carcinogenesis induced by 2-amino-3-methylimidazo[4,5-f]quinoline, a food mutagen. *Cancer Research*, *53*, 3914–3918.

Reiss, J. (1994). Influence of different sugars on the metabolism of the tea fungus. *Zeitschrift fur Lebensmittel-Untersuchung und -Forschung*, *198*, 258–261.

Ried, K., Toben, C., & Fakler, P. (2013). Effect of garlic on serum lipids: An updated metaanalysis. *Nutrition Reviews*, *71*, 282–299. doi: 10.1111/nure.12012.

Rodríguez-Mateos, A., Vauzour, D., Krueger, C. G., Shanmuganayagam, D., Reed, J., & Calani, L. (2014). Bioavailability, bioactivity and impact on health of dietary flavonoids and related compounds: An update. *Archives of Toxicology*, *88*, 1803–1853. doi: 10.1007/s00204-014-1330-7.

Rosado, J. L., Solomons, N. W., & Allen, L. H. (1992). Lactose digestion from unmodified, low-fat and lactose-hydrolyzed yogurt in adult lactose maldigesters. *European Journal of Clinical Nutrition*, *46*, 61–67.

Roy, B., Kala, C. P., Farooquee, N. A., & Majila, B. S. (2004). Indigenous fermented food and beverages: A potential for economic development of the high altitude societies in Uttaranchal. *Journal of Human Ecology*, *15*, 45–49.

Ryu, S. H., Jeon, Y. S., Kwon, M. J., Moon, J. W., Lee, Y. S., & Moon, G. S. (1997). Effect of kimchi extracts to reactive oxygen species in skin cell cytotoxicity. *Journal of the Korean Society of Food Science and Nutrition*, *26*, 814–821.

Saarela, M., Lahteenmaki, L., Crittenden, R., Salminen, S., & Mattila-Sandholm, T. (2002). Gut bacteria and health foods—The European perspective. *International Journal of Food Microbiology*, *78*, 99–117.

Saloff-Coste, C. J. (1996). Kefir. *Danone World Newsletter* (11).

Satish Kumar, R., Kanmani, P., Yuvaraj, N., Paari, K. A., Pattukumar, V., & Arul, V. (2013). Traditional Indian fermented foods: A rich source of lactic acid bacteria. *International Journal of Food Sciences and Nutrition*, *64*, 415–428.

Scalbert, A., Manach, C., Morand, C., Rémésy, C., & Jiménez, L. (2005). Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition*, *45*, 287–306. doi: 10.1080/1040869059096.

Schaafsma, G. J., Dekker, P. R., & de Ward, H. (1988). Nutritional aspects of yogurt. Bioavailability of essential minerals and trace elements. *Netherlands Milk and Dairy Journal*, *42*, 135–146.

Semwal, A., Kaushik, S., Bhatt, S. P., & Negi, A. (2011). Antibacterial activity of *Diplazium esculentum* (Retz.) Sw. *Pharmacognosy Journal*, *21*(4), 74–76.

Seong, G. U., Hwang, I. W., & Chung, S. K. (2016). Antioxidant capacities and polyphenolics of Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*) leaves. *Food Chemistry*, *199*, 612–618. doi: 10.1016/j.foodchem.2015.12.066.

Shackelford, L. A., Rao, D. R., Chawan, C. B., & Pulusani, S. R. (1983). Effect of feeding fermented milk on the incidence of chemically induced colon tumors in rats. *Nutrition and Cancer*, *5*, 159–164.

Shahani, K. M., & Chandan, R. C. (1979). Nutritional and healthful aspects of cultured and culture-containing dairy foods. *Journal of Dairy Science*, *62*, 1685–1694.

Shantha, N. C., Ram, L. N., O'Leary, J., Hicks, C. L., & Decker, E. A. (1995). Conjugated linoleic acid concentrations in dairy products as affected by processing and storage. *Journal of Food Sciences*, *60*, 695–698.

Sharif, M., Moridnia, M., Mortazavi, D., Salehi, M., Bagheri, M., & Sheikhi, A. (2017). Kefr: A powerful probiotic with anticancer properties. *Medical Oncology*, *34*, 183. doi: 10.1007/s12032-017-1044-9.

Sharp, M. D., McMahon, D. J., & Broadbent, J. R. (2008). Comparative evaluation of yogurt and low-fat Cheddar cheese as delivery media for probiotic *Lactobacillus casei*. *Journal of Food Science*, *73*(7), M375–M377.

Singh, J., Upadhyay, A. K., Prasad, K., Bahadur, A., & Rai, M. (2007). Variability of carotenes, vitamin C, E and phenolics in *Brassica* vegetables. *Journal of Food Composition and Analysis*, *20*, 106–112. doi: 10.1016/j.jfca.2006.08.002.

Slimestad, R., Fossen, T., & Vagen, I. M. (2007). Onions: A source of unique dietary flavonoids. *Journal of Agricultural and Food Chemistry*, *55*, 10067–10080. doi: 10.1021/jf0712503.

Smacchi, E., & Gobbetti, M. (1998). Peptides from several Italian cheeses inhibitory to proteolytic enzymes of lactic acid bacteria: *Pseudomonas fluorescens* ATCC 948 and to the angiotensin I-converting enzyme. *Enzyme and Microbial Technology*, *22*, 687–694.

Solis-Pereyra, B., Aattouri, N., & Lemonnier, D. (1997). Role of food in the stimulation of cytokine production. *The American Journal of Clinical Nutrition*, *66*(Suppl.), 521S–525S.

Sreeramulu, G., Zhu, Y., & Knol, W. (2000). Kombucha fermentation and its antimicrobial activity. *Journal of Agricultural and Food Chemistry*, *48*, 2589–2594.

Srinivasan, K. (2014). Antioxidant potential of spices and their active constituents. *Critical Reviews in Food Science*, *54*, 352–372. doi: 10.1080/10408398.2011.585525.

Stabler, S. N., Tejani, A. M., Huynh, F., & Fowkes, C. (2012). Garlic for the prevention of cardiovascular morbidity and mortality in hypertensive patients. *Cochrane Database of Systematic Reviews*, *8*, CD007653. doi: 10.1002/14651858.cd007653.pub2.

Stanton, C., Desmond, C., Coakley, M., Collins, J. K., Fitzgerald, G., & Ross, R. P. (2003). Challenges facing development of probiotic-containing functional foods. In E. R. Farnworth (Ed.), *Handbook of fermented functional foods* (pp. 27–58). Boca Ranton, FL: CRC Press.

Stasiak, L., & Blazejak, S. (2009). Acetic acid bacteria, perspectives of application in biotechnology, a review. *Polish Journal of Food and Nutrition Sciences*, *59*, 17–23.

Steinkraus, K. (1996). *Handbook of indigenous fermented foods*. New York, NY: Marcel Decker.

Stoilova, I., Krastanov, A., Stoyanova, A., Denev, P., & Gargovaa, S. (2007). Antioxidant activity of a ginger extract (*Zingiber officinale*). *Food Chemistry*, *102*, 764–770. doi: 10.1016/j.foodchem.2006.06.023.

Sveje, M. (2007). Probiotic and prebiotics improving consumer health through food consumption. *Nutracoss*, 28–31.

Tamang, J. P., Watanabe, K., & Holzapfel, W. H. (2016). Review: Diversity of microorganisms in global fermented foods and beverages. *Frontiers in Microbiology*, *7*, 377. doi: 10.3389/fmicb.2016.00377.

Tamime, A. Y. (2002). Fermented milks: A historical food with modern applications—A review. *European Journal of Clinical Nutrition*, *56*, S2–S15.

Tedesco, I., Carbone, V., Spagnuolo, C., Minasi, P., & Russo, G. L. (2015). Identification and quantification of flavonoids from two southern Italian cultivars of *Allium cepa* L., Tropea (Red Onion) and Montoro (Copper Onion), and their capacity to protect human erythrocytes from oxidative stress. *Journal of Agricultural and Food Chemistry*, *63*, 5229–5238. doi: 10.1021/acs.jafc.5b01206.

Teyssier, C., & Hamdouche, Y. (2016). *Acetic acid bacteria: Prospective applications in food biotechnology*. Northwestern, FL: CRC Press.

Troitino, C. (2018). Kombucha-101-demystifying-the-past-presentand-future-of-the-fermented-tea-drink. Accessed 12.07.18 (/#261ea4684ae2). https://www.forbes.com/sites/christinatroitino/2017/02/01/.

Umadevi, M., Pushpa, R., Sampathkumar, K. P., & Bhowmik, D. (2012). Rice-traditional medicinal plant in India. *Journal of Pharmacognosy and Phytochemistry*.

Upretia, P., & Metzge, L. E. (2007). Influence of calcium and phosphorus, lactose, and salt-to-moisture ratio on Cheddar cheese quality: pH changes during ripening. *Journal of Dairy Science*, *90*(1), 1–12.

USDA, Agricultural Research Service. (2013). *USDA national nutrient database for standard reference, release 26*. Nutrient Data Laboratory. Available from: http://www.ars.usda.gov/ba/bhnrc/ndl (cited 24 January 2014).

Valenzuela, A. (2004). El consumo de té y la salud: Características y propiedades benéficas de esta bebida milenaria. *Revista Chilena De Nutrición*, *31*, 72–82.

Varzakas, T., Zakynthinos, G., Proestos, C., & Radwanska, M. (2017). Fermented vegetables. *Minimally processed refrigerated fruits and vegetables*. Springer US.

Vesa, T. H., Marteau, P., & Korpela, R. (2000). Lactose intolerance. *Journal of the American College of Nutrition*, *19*, 165S–175S.

Villarreal-Soto, S. A., Beaufort, S., Bouajila, J., Souchard, J. P., & Taillandier, P. (2018). Understanding Kombucha tea fermentation: A review. *Journal of Food Sciences*, *83*(3), 580–588.

Vina, I., Semjonovs, P., Linde, R., & Deninja, I. (2014). Current evidence on physiological activity and expected health effects of kombucha fermented beverage. *Journal of Medicinal Food*, *17*(2), 179–188.

Wang, H., Livingston, K. A., Fox, C. S., Meigs, J. B., & Jacques, P. F. (2013). Yogurt consumption is associated with better diet quality and metabolic profile in American men and women. *Nutrition Research*, *33*, 18–26.

Watanabe, M., Musumi, K., & Ayugase, J. (2011). Carotenoid pigment composition, polyphenol content, and antioxidant activities of extracts from orange-colored Chinese cabbage. *LWT – Food Science and Technology*, *44*, 1971–1975. doi: 10.1016/j.lwt.2011.04.010.

Watawana, M. I., Jayawardena, N., Gunawardhana, C. B., & Waisundara, V. Y. (2015). Health, wellness, and safety aspects of the consumption of kombucha. *Journal of Chemistry*, *1*, 1–11.

Watson, E. (2018). *Fermentation on fire: US retail sales of kombucha and other fermented beverage surged 37.4% in 2017*. . https://www.foodnavigatorusa.com/Article/2018/02/13/ Accessed 12.07.18.

Whigham, L. D., Cook, M. E., & Atkinson, R. L. (2000). Conjugated linoleic acid: Implications for human health. *Pharmacological Research*, *42*, 503–510.

Wilkinson, M. G., Meehan, H., Stanton, C., & Cowan, C. (2001). Marketing cheese with a nutrient message. *Bulletin—International Dairy Federation*, *363*, 39–45.

Wills, R. B. H., & Rangga, A. (1996). Determination of carotenoids in Chinese vegetables. *Food Chemistry*, *56*, 451–455. doi: 10.1016/0308-8146(95)00226-X.

Wollowski, I., Ji, S., Bakalinsky, A. T., Neudecker, C., & Pool-Zobel, B. L. (1999). Bacteria used for the production of yogurt inactivate carcinogens and prevent DNA damage in the colon of rats. *The Journal of Nutrition*, *129*, 77–82.

Yadava, R. N., & Reddy, V. M. S. (2003). Anti-inflammatory activity of novel flavonol glycoside from *Bauhinia variegata* L. *Natural Product Research*, *17*(3), 165–169.

Yang, D., Liu, X., Liu, M., Chi, H., Liu, J., & Han, H. (2015). Protective effects of quercetin and taraxasterol against H_2O_2-induced human umbilical vein endothelial cell injury in vitro. *Experimental and Therapeutic Medicine*, *10*, 1253–1260. doi: 10.3892/etm.2015.2713.

Yang, H. J., Park, S., Pak, V., Chung, R. K., & Kwon, D. Y. (2011). *Fermented soybean products and their bioactive compounds*. InTechOpendoi: 10.5772/10670.

Yang, X., Hub, C., Xiu, Z., Jiang, A., Yang, X., Sarengaowa, et al. (2019). Microbial dynamics and volatilome profiles during the fermentation of Chinese northeast sauerkraut by *Leuconostoc mesenteroides* ORC 2 and *Lactobacillus plantarum* HBUAS 51041 under different salt concentrations. *Food Research International*, *130*, 108926. doi: 10.1016/j.foodres.2019.108926.

Yuvaraj, K. P. (2019). Medicinal value of ancient Tamilnadu authentic food—A detail study. *International Journal of Latest Technology in Engineering Management & Applied Science*, *VIII*(IV), 2278–2254.

Zemel, M. B., Richards, J., Mathis, S., Milstead, A., Gebhardt, L., & Silva, E. (2005). Dairy augmentation of total and central fat loss in obese subjects. *International Journal of Obesity*, *29*, 391–397.

Zheng, W., Blot, W. J., Shu, X. -O., Gao, Y. -T., Ji, B. -T., Ziegler, R. G., et al. (1992). Diet and other risk factors for laryngeal cancer in Shanghai, China. *American Journal of Epidemiology*, *136*, 178–191.

Zoecklein, W., Fugeslang, K., Gump, B., & Nury, F. (1999). *Wine analysis and production*. New York, NY: Aspen Publishers.

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Chapter 28

Main Technological Challenges Associated With the Incorporation of Probiotic Cultures into Foods

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Abbreviations

Lb. *Lactobacillus*
B. *Bifidobacterium*
Bc. *Bacillus*
CFU colony forming unit
EPS exopolysaccharides
GIT gastrointestinal tract
Sacch. *Saccharomyces*
Strept. *Streptococcus*
Lc. *Lactococcus*
Ent. *Enterococcus*
Pc. Pediococcus
FOS fructooligosaccharides
GOS galactooligosaccharides

1 Introduction to probiotic-containing functional foods

The term "functional food" first appeared in Japan in the 1980s, making a clear reference to the ability of some foods to improve the well-being of the consumer (Roberfroid, 2000). It was not an original concept at all, since Hippocrates, the father of medicine, had already understood the importance of nutrition to our health and psychophysical well-being already 400 before Christ. Hippocrates coined the statement "Let food be your medicine and medicine be your food," which is still one of the greatest medical advices ever suggested to human populations. The modern consumer, now more than ever, has embraced this suggestion, profoundly changing their food choices from foods that can simply meet nutritional needs toward foods that can improve health. This attitude finds its expression in the definition of functional foods given by The European Commission Concerted Action on Functional Food Science in Europe, which says "A food can be regarded as 'functional' if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease" (Diplock et al., 1999). The benefits attributed to functional foods include the improvement of general body conditions, reduction of the risk of specific diseases, and potential use for prevention and treatment of illnesses (Bigliardi & Galati, 2013). Functional foods are similar in appearance to conventional foods consumed in a normal diet and contain bioactive components whose physiological activity is scientifically proven. The main categories of functional foods include basic foods, that is, those that naturally contain one or more bioactive components, fortified foods, that is, those in which the native bioactive level is modified or concentrated, and processed foods, which are intentionally added with a bioactive

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00028-9
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during the production process. Bioactive ingredients that confer the character of functionality to foods can be vitamins, antioxidants, fibers, minerals, omega-3, and probiotic cultures.

According to the currently most accredited definition, probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (Hill et al., 2014). This concept constitutes a direct inheritance from the observations of the Nobel Laureate Élie Metchnikoff, who at the beginning of the 20th century hypothesized a correlation between the consumption of fermented milk containing *Lactobacillus* and the longevity of a population of Bulgarian farmers (Metchnikoff, 1908). Based on his studies, he suggested the possibility of modifying the intestinal flora by replacing dangerous microorganisms with useful ones present in some foods. Since then, enormous research efforts have been made to isolate and characterize new microbial strains capable of performing probiotic activities. The beneficial effects attributed to probiotic cultures include, among others, prevention and treatment of allergies and atopic dermatitis, irritable bowel syndrome, intestinal inflammation, diarrhea and constipation of viral and bacterial origin, immunomodulation, and improvement of oral health (Moslehi-Jenabian, Pedersen, & Jespersen, 2010). The most commonly used probiotics belong to the genera *Lactobacillus* (*Lb. casei, Lb. acidophilus, Lb. rhamnosus, Lb. johnsonii* among others) and *Bifidobacterium* (*B. infantis, B. longum, B. bifidum, B. breve*), mainly due to the fact that these are considered safe, simple to manage on an industrial level (which applies above all to *Lactobacillus*), and of intestinal origin, not to mention that there is strong scientific evidence relating to their beneficial effect on health. Other important probiotic species, although less frequently used, are *Enterococcus faecium, Bacillus* spp., and *Saccharomyces boulardii* (Felis, Dellaglio, & Torriani, 2009).

2 Probiotic foods on the market

Based on the raised awareness of the consumer toward the role of diet on health and the increasing scientific evidence relating to the properties of probiotic microorganisms, the food industry has developed a very large number of functional foods containing probiotic microorganisms that can meet the needs of more diverse consumer demands. Consequently, the market of probiotic foods has become a leading one in recent years. It was estimated that in 2017, the share of consumers interested in introducing more probiotic foods into their diet was around 38% (Sloan, 2018).

Dairy foods are undoubtedly the most popular and best-selling probiotic food category worldwide, probably thanks to the fact that milk-based products are considered intrinsically healthy and rich in nutrients, which can guarantee the growth of probiotics. Probiotic yoghurt is the leading product (about 35% of the products overall), followed by cultured drinks, kefir and cheese, frozen dairy desserts and cream-based dairy desserts, and infant formula (Innocente et al., 2016; Saarela, 2018). Although fermented products (yoghurt and cheese) are those most commonly added with probiotic cultures, there are also several examples of unfermented dairy matrices, such as ice cream, butter, and sweet acidophilus milk, in which the addition of probiotics has proven to have some clinical efficacy against conditions such as lactose intolerance, constipation, and excessive levels of salivary *Streptococcus mutans* (Koebnick, Wagner, Leitzmann, Stern, & Zunft, 2003; Mahantesha et al., 2015). Cheese whey, which is particularly rich in nutrients, has also proven to be a suitable vehicle for probiotic microorganisms, both in the form of fermented or unfermented beverages and whey cheeses (Suomalainen et al., 2006). It should also be stressed that the functional use of whey, which is a by-product of cheesemaking that is often discarded, takes on special significance for the environmental sustainability of the entire dairy supply chain.

Although meat is an excellent source of protein of high biological value and its consumption contributes to enriching the diet with easily assimilable iron, some consumers associate this product and its derivatives with a negative health image. The use of meat as a carrier of probiotic microorganisms, therefore, might represent an interesting strategy to be explored by the industry for the production of healthy food. Although the use of probiotic microorganisms in meat has been studied above all from a microbiological, technological, and sensory point of view, some data indicate that the consumption of meat with added probiotics is also capable of inducing positive effects on the immune response and blood level of cholesterol and triglycerides (Khan et al., 2011).

Given the increasing incidence of allergies to milk proteins, lactose intolerance, as well as specific diet choices that partially or completely exclude food of animal origin, the market has responded by making many plant-based probiotic products available to the consumer. The development of such food products helps reduce cardiovascular risk related to high cholesterol level, and satisfies the eating habits of various ethnic groups. It should also be borne in mind that raw materials of plant origin often contain prebiotic substances, which can stimulate the growth of probiotic microorganisms both during the processing steps and during storage (Senaka Ranadheera, Evans, Adams, & Baines, 2012).

Cereals are produced and consumed worldwide, which makes cereal-based probiotics easily accessible to a large part of the population. Cereals, in particular barley and oats, are very important raw materials for the production of functional foods, as well as being an important source of fibers, which have various beneficial physiological effects on the intestinal microflora. Flake cereals and cereal bars have been used as a direct carrier of dried probiotic cultures. Moreover, new

cereal-based probiotic drinks have been developed, containing malt, wheat, barley, and oat, which have proven to be able to support microbial growth and improve tolerance to bile salts. Furthermore, the ability to stimulate the growth of intestinal *Bifidobacterium* was highlighted (Hajduk et al., 2009; Mitsou, Panopoulou, Turunen, Spiliotis, & Kyriacou, 2010).

As for legumes, soybeans are the most commonly ones associated with the addition of probiotic microorganisms. This approach contributes to mitigating some nutritional and sensory problems associated with the consumption of soy products, such as the presence of indigestible oligosaccharides (e.g., raffinose and stachyose) that cause intestinal disorders, and unpleasant beany flavor, which significantly limit the consumption of these products (Shi et al., 2015). Soymilk sustains the growth of various lactic acid bacteria and bifidobacteria, which allow reducing the concentration of indigestible oligosaccharides by releasing sucrose, fructose, and galactose through fermentation (Wang, Yu, & Chou, 2006). Another reason encouraging the use of probiotics in soy is the fact that these microorganisms produce the β-glucosidase and β-galactosidase necessary for the conversion of isoflavone glycosides into their bioactive aglycone forms, which have antioxidant, antihypertensive, and anticarcinogenic effects (Otieno, Ashton, & Shah, 2006). The consumption of soy-based probiotic products has been shown to have various clinical effects, including the reduction of some oxidative stress factors in diabetic patients, improvement of the lipid profile in the case of hypercholesterolemia and of the skin condition in menopausal women (Cavallini et al., 2016).

The use of fruit and vegetables as vehicles for probiotic microorganisms is also quite attractive, as these products are generally perceived as healthy by the consumer and have an appealing sensory profile. They also have marked functional properties *per se*, as they are rich in fibers, vitamins, minerals, flavonoids, and antioxidants. Enrichment with probiotic cultures is carried out either by impregnating fruit or vegetables pieces through osmotic dehydration or vacuum impregnation techniques, or by directly adding the microbial cells to juices. In the latter case, a fermentation step may occur. Only a few clinical trials have been performed on fruit and vegetables enriched with probiotics, and it has been recently observed that fruit juice enriched with *Lb. rhamnosus* GG has shown a reduction of the symptoms of viral colds (Kumpu et al., 2015).

Given the strong consumer demands, the most innovative suppliers have placed on the market several products containing probiotics, including water, chocolate, tea, spreads, and even bars and sweet fingers, which contribute to increasing the offer for the most demanding consumers (Hernández-Barrueta et al., 2020; Santos et al., 2020).

3 Factors affecting probiotics' viability

Although some functional activities can also be attributed to inactivated or killed microbial cells or fragments thereof (Barros et al., 2020), in general, the health benefits are ensured in the presence of live and viable probiotic microorganisms. Several international authorities agree that the microbial viability must be quite high, with minimum values of at least 10^6–10^7 CFU (colony forming unit)/g, so that the intake of a portion of about 100 g allows the ingestion of 10^8–10^9 probiotic cells (Jayamanne & Adams, 2006). Since the health effects depend on the conditions of the product at the time of consumption, the maintenance of microbial viability must be ensured over its entire shelf-life. Probiotic microorganisms are quite sensitive, and the number of viable bacteria can decrease by 1–2 log CFU/g or even more during production and storage. Their viability in food is strongly endangered by various factors, including environmental features of the food matrix, processing treatments applied, possible presence of starter microflora (in the case of fermented food), conditions and duration of storage, as well as gastrointestinal tract (GIT) transit (Fig. 28.1).

3.1 Food matrix features

The carrier matrix has a significant effect on the quality of probiotic products, as its composition and characteristics can alter the viability and effectiveness of probiotics. One of the main obstacles is represented by the acid pH, since lactobacilli have an optimal growth pH of 5.5–6.0, and bifidobacteria of 6.0–7.0 (De Vuyst, 2000). Acid pH, which is relevant for fermented foods, fruit, and vegetables, leads to an increase in the concentration of organic acids in the undissociated form, therefore increasing the antimicrobial effect of these acids. Furthermore, at low pH values (below 4.5), cells need a lot of energy to keep the intracellular pH constant, which seizes ATP (adenosine triphosphate) from other cellular needs causing cell death. Low pH (about 3.5) was shown to be the main culprit in the loss of viability of *Lb. rhamnosus, Lb. plantarum,* and *Lb. casei* in cherry juice, especially during refrigerated storage (Nematollahi, Sohrabvandi, Mortazavian, & Jazaeri, 2016). In fermented milk, *Lb. acidophilus* tolerates acid conditions better than bifidobacteria, proving the careful selection of the strains to be used in each probiotic food to be crucial (Tamime, Saarela, Søndergaard, Mistry, & Shah, 2006).

Water intended both as moisture and a_w also plays a determining role in the survival of probiotics in food. The a_w value, which expresses the availability of water for microbial growth, varies from 0.15 in dry foods to 0.99 in fresh products. At a_w above 0.3, there is an important loss of viability, while in low moisture or intermediate moisture foods

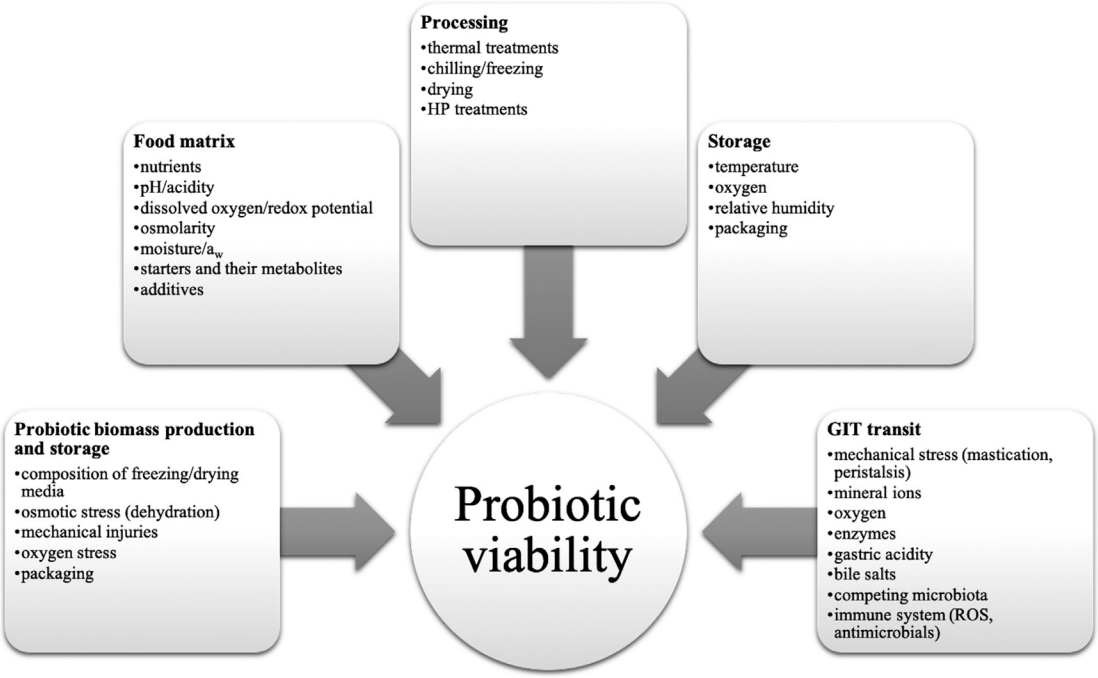


FIGURE 28.1 Main factors affecting probiotics' viability in foods.

probiotics are stable for prolonged times, as it happens in chocolate, peanut butter, cereals, and dried-fruit paste (Finn, Condell, McClure, Amézquita, & Fanning, 2013). In these food categories, low a_w values are the key factor for the long-term maintenance of cell viability, which are metabolically inactive. *Lb. rhamnosus* exhibited a viability loss of less than 1 log CFU/g in peanut butter (a_w about 0.35) stored at 25°C for 27 weeks (Klu, Williams, Phillips, & Chen, 2012), and similar result has been observed for *Bifidobacterium* (Klu, Phillips, & Chen, 2014). The exposure to humidity during storage has a negative effect on viability, in fact, at increasing levels of moisture and a_w the survival rate of probiotics decreases, also due to the possible growth of other microorganisms and fungi, as well as to unwanted chemical reactions.

Closely connected to the a_w value is the food osmolarity, particularly high in foods containing high amounts of sugar or salt, such as fruit purees, jams, or aged cheeses. Sugar and salt greatly influence the growth and survival of probiotics in food as their presence increases osmotic pressure. *Lb. casei* has shown slow growth in the presence of high glucose concentrations, as well as bifidobacteria, while *Lb. plantarum* seems to be more resistant (Homayouni, Azizi, Ehsani, Yarmand, & Razavi, 2008a). The tolerance to osmotic stress is strain-specific and depends on the phospholipidic composition of the membrane. Salt is widely used in food to prolong its shelflife, but at high concentrations (about 4%–6%), it can put the viability of probiotics at risk. However, low salt or sugar concentrations can stimulate the survival of *Lactobacillus* to freeze-drying (Waako, Polak-Berecka, & Gustaw, 2013).

Since most probiotic species are strictly anaerobic, the presence of dissolved oxygen in the matrix is a critical factor for their survival in foods, as hydrogen peroxide is formed through different mechanisms both at intracellular and extracellular level. In fact, probiotic bacteria are practically devoid of an oxygen scavenging system, which causes the reduction of oxygen to hydrogen peroxide. The toxicity of hydrogen peroxide is due to its ability to block fructose-6-phosphofructoketolase, a key enzyme for sugar metabolism. In addition, superoxide and hydroxyl radicals are formed in the presence of dissolved oxygen due to the oxidation of membrane lipids in the cell, which induces DNA damage and causes cell death (Önneby et al., 2013). Different processing steps can contribute to the enrichment of the matrix in dissolved oxygen, such as homogenization, cooling after heat treatment and mixing with ingredients. Although all probiotics are anaerobic, oxygen tolerance varies enormously between genera and species. Indeed, bifidobacteria tend to be more sensitive than lactic acid bacteria. In the presence of oxygen, the most sensitive bacteria undergo changes in the profile of membrane fatty acids and show an elongation of the lag phase and a strong reduction in the amount of lactate produced (Ahn, Hwang, & Park, 2001; Talwalkar & Kailasapathy, 2003).

The presence of food preservatives and additives can adversely affect cell growth and metabolism of probiotics. In fermented meats, the presence of sodium nitrite or bacteriocins induces, in addition to their acid pH, considerable stress

on probiotics (Lee et al., 2008). In yoghurt and fermented milk, sweeteners, flavorings, and artificial colorings, volatile compounds of microbial origin (diacetyl, acetaldehyde, and acetoin) and bacteriocins can interfere with microbial viability (Tripathi & Giri, 2014). Aspartame, a sweetener used in fermented milk-based drinks, has a certain antimicrobial activity when used at a concentration of 0.12%. While natural dyes such as carmine, turmeric, and bixin have no effect on the viability of probiotics, some flavoring-coloring commercial mixtures are inhibiting even when used at the recommended concentrations (Vinderola, Mocchiutti, & Reinheimer, 2002).

In some cases, the solid matrix of the food itself is partly responsible for a certain degree of protection of microbial viability. This is the case of cheese, in which the high lipid content, low redox potential, and high buffering capacity contribute to protecting probiotics both during the ripening and the GIT transit. Furthermore, the dry matter adsorbs the hydrogen ions, causing an increase in the amount of organic acids in the undissociated form and consequently reducing their toxicity toward probiotics (de Almeida et al., 2018).

3.2 Processing conditions

During the production of food containing probiotics, microorganisms can be exposed to thermal stress at various times. When heat treatments are used to inactivate pathogenic and spoiler microorganisms in raw materials, thermal stress on probiotics can be easily avoided by inoculating them downstream of the heat treatment or by using probiotic species other than lactic acid bacteria and bifidobacteria, such as spores of the genus *Bacillus*. *Bc. coagulans* has been successfully used in the processing of various foods such as banana muffins and waffles, brewed coffee, chocolate fudge frosting, and hot fudge topping, and the loss of viability has been less than 1 log CFU/g in all cases (Majeed et al., 2016).

Even during fermentation, probiotics may undergo thermal stress. During the production of yoghurt, milk is fermented at temperatures above 40°C–42°C, which can be harmful to probiotics. In the case of *Lb. acidophilus* and *Bifidobacterium* BB-12 in yoghurt, the incubation temperature of 44°C caused a decrease in viability, which did not occur at 37°C. At the highest fermentation temperatures (e.g., 45°C), *Lb. delbrueckii* subsp. *bulgaricus* becomes the dominant species and produces a large amount of lactic acid, hydrogen peroxide, and sometimes bacteriocins, which causes probiotic growth to stop (Mortazavian et al., 2006).

Freezing represents one of the most critical steps in some probiotic foods, such as ice cream. The phenomena occurring at subzero temperatures have a great influence on microbial viability. As the temperature drops and the portion of frozen water increases, the a_w value of the liquid solution decreases. Then, by osmosis, a part of water diffuses outside the cell, which dehydrates, and the extracellular water freezes. All this, together with the progressive lowering of the temperature, determines the so-called cold shock, which causes cell death. The mechanical stress due to ice crystals damages the membranes, the low temperatures shocks the cells, the (harmful) solutes concentrate, and the cells become dehydrated. In probiotic ice cream, *Lb. rhamnosus* undergoes a loss of viability during freezing of about 1.8 log CFU/g, probably due to a combined effect of temperature lowering and oxygen incorporation during mixing (Marino et al., 2017).

3.3 Competition with starter cultures

Most of the probiotic foods on the market derive from fermented foods already containing starter cultures. Probiotics alone grow slowly in the raw materials used, for example, milk, and generate final products with unsatisfactory sensory characteristics (Tamime, Saarela, Søndergaard, Mistry, & Shah, 2006). Starters can compete with probiotics by reducing their viability, while the opposite rarely happens. In the case of bifidobacteria, one possible explanation of the phenomenon is their rapid growth in the first few hours of fermentation, followed by a stop at low final viabilities (Samona & Robinson, 1994), probably due to their limited proteolytic activity. When starters are poorly proteolytic, they compete with probiotics to assimilate the small amount of amino acids and peptides that are present in milk, and this can lead to the loss of viability of probiotics. The same happens with the available carbon sources, which are present in high concentrations at the beginning of fermentation, thus leading to competition and rapid use by the fastest microorganisms, that is, the starters (Anandharaj, Rani, & Swain, 2018).

Another cause of probiotic inhibition is the production of hydrogen peroxide by some starters, such as *Lb. delbrueckii*, and this can lead to an inhibition of probiotics during the storage of yoghurt. Peroxidase-producing probiotics are therefore less sensitive to this type of biological competition (Shimamura et al., 1992). Even the production of bacteriocins by starters can cause the inactivation of probiotics, which can occur in the case of cheese, while it has not been observed in yoghurt. The possible interactions between starters and probiotics also include the production of diffusible chemical compounds that are involved in the mechanisms of *quorum sensing* (Anandharaj, Rani, & Swain, 2018).

3.4 Storage conditions

The viability of probiotic microorganisms in food can decrease in a more or less accentuated way during storage. Room temperature storage can be detrimental to probiotics. *Lb. rhamnosus* in peach jam survived up to 78 days at 5°C, while at 25°C the loss of viability was unacceptable already after 45 days (Randazzo, Pitino, Licciardello, Muratore, & Caggia, 2013). In general, lactobacilli better tolerate the low temperatures than bifidobacteria. In yoghurt, *Lb. acidophilus* maintained maximum viability for 20 days of storage at 2°C, while *B. lactis* at 8°C (Mortazavian et al., 2007). In addition to the factors already discussed, the type of packaging can also significantly contribute to maintaining the viability of probiotics. In this regard, oxygen permeability of the packaging is fundamental, given the anaerobiosis of most probiotics (Ladero & Sánchez, 2017). It should be highlighted that the specific characteristics and the thickness of the film matrix, as well as the environmental temperature and relative humidity may affect the permeability of the packaging material, thus influencing probiotics' viability during storage. The materials that best protect probiotics are glass, oxygen-barrier packaging, multilayer packaging with selective permeability, and active packaging containing oxygen scavengers (Miller, Nguyen, Rooney, & Kailasapathy, 2003). In green Spanish-style Manzanilla olives packaged in different conditions, *Lb. pentosus* viability decreased over time, and the highest survival after 8 months at 20°C was observed in plastic pouches under nitrogen atmosphere and glass jars with brine (Rodríguez-Gómez, Romero-Gil, García-García, Arroyo-López, & Garrido-Fernández, 2016). Carbonation of liquid foods, for example, milk, partially displaces the oxygen and may contribute to probiotics' survival (Hotchkiss, Werner, & Lee, 2006).

3.5 GIT transit

To exert the health effects they are selected for, probiotics must survive the GIT transit, which depends first on their tolerance to acidity and bile. This is an intrinsic feature of the strain, which however can be influenced by the type food carrier. In the stomach, the main factors that can reduce microbial viability are the low pH and antimicrobial activity of pepsin, and the food normally remains in the stomach 2–4 h. Subsequently, probiotics must survive in the environment of the small intestine at a pH of about 8.0, where they are exposed to the antimicrobial activity of bile salts and pancreatin (Kailasapathy, 2006).

The food may contain metabolizable nutrients or buffering substances whose presence can positively influence the survival of probiotics during GIT transit. In simulated gastric conditions, probiotics in dairy products performed better than the same strains in lyophilized capsules (Fredua-Agyeman & Gaisford, 2015). Hence, the type of carrier food is of primary importance in determining the ability of probiotics to reach the target site. *Lb. acidophilus, B. animalis* subsp. *lactis*, and *Propionibacterium jensenii* were more protected from gastric acidity and bile when they were ingested with ice cream than with white yoghurt and fruit, and a similar observation was made in whey-based cheeses. Similarly, the type of food influenced the ability of probiotics to adhere to cells of intestinal origin (Ranadheera, Evans, Adams, & Baines, 2012). In the case of *Lb. helveticus, Lb. rhamnosus, B. longum*, and *Sacch. boulardii*, ingestion in milk with 1% fat and oatmeal-milk gruel was better than apple juice and spring water, indicating that the fat content is more important than the protein content in terms of protection during the GIT transit (Tompkins, Mainville, & Arcand, 2011). As for the matrices of plant origin, fermented carrot juice has been shown to offer *Lb. rhamnosus* protection in a similar way to dairy products, unlike rice cream, which offered less protection from gastric acidity (Sagheddu et al., 2018). *Lb. paracasei* conveyed in artichokes was better, and in a human study, the microorganism was recovered from stools, indicating the ability to survive the GIT transit (Valerio et al., 2006). Similarly, a cheese matrix allowed a better fecal recovery of bifidobacteria and lactobacilli than a freeze-dried capsule or yoghurt, and in the case of propionibacteria, survival was higher in the yoghurt matrix than cheese and capsules (Saxelin et al., 2010).

4 Intervention strategies

As discussed so far, adding probiotics to foods poses many challenges to ensuring the survival of microorganisms during production, storage, and GIT transit. These challenges have offered research many starting points for the development of technological and biotechnological strategies aimed at improving the quality of probiotic foods. Consequently, many advances have been made in the field of microbial strain selection, in producing microbial biomass, in the use of new ingredients and in the development of suitable encapsulation techniques.

4.1 Strain selection and inoculation condition

Strain selection is one of the most important factors that determine the effectiveness of probiotic food. The criteria that guide the selection of the strains are of four types: technological criteria (relating, e.g., to the ability to grow in a specific

food matrix or to tolerate a process or storage phase), safety criteria (e.g., enterotoxin production, hemolytic activity, or presence of virulence factors), functional criteria (e.g., tolerance to acid and bile, adhesion to the mucosal surface), and physiological criteria (e.g., anticancer activity, anticholesterol activity, or immunostimulatory activity). The selection of a large number of strains and the evaluation of numerous characteristics all together can be facilitated by the use of a step-by-step approach, in which a sequence of tests are carried out to progressively reduce the number of probiotic candidates, or by the use of algorithms and heat-map analysis, which allow clustering of the strains and identification of those showing the highest values for all assessed functional phenotypes (Fernandez-Pacheco Rodríguez, Arévalo-Villena, Zaparoli Rosa, & Briones Pérez, 2018).

For the survival of the strains in food, the criteria to be taken into consideration are certainly the technological ones, and many authors report that these characteristics are strictly strain-specific. By way of example, lactobacilli are more resistant and more suitable for the industrial use than bifidobacteria, as they better tolerate acidity and the presence of oxygen; the latter, on the other hand, require the presence of specific growth factors (Tripathi & Giri, 2014). Therefore, it is necessary to know in depth the growth characteristics and the nutritional needs of the strains, to highlight which possible foods can be added with specific probiotics. The ability to grow on specific food substrates is phenotypically verified, but it can also be predicted based on the presence of specific genes harbored for utilizing certain simple or complex sugars present in specific food matrices (Månberger et al., 2020).

Traditionally, the selection is carried out based on appropriate phenotypic tests; however, the enormous recent technological advances made in the context of high-throughput *omic* technologies - that is, genomics, transcriptomics, proteomics, and metabolomics - have allowed to boost those sectors of scientific research that deal with selecting new and more performing probiotic strains. In addition to being the best tool for identifying strains, genomics can help in detecting strains that have desirable or undesirable characteristics, and therefore, its use is likely to become a standard procedure in the coming years. High-throughput transcriptomics and proteomics will enable rapid screening of specific functional properties, as well as more efficient *in vitro* and *in vivo* tests. Similarly, *omic* technologies have been helping us to better understand the complexity of the human microbiome and to highlight a wide range of new probiotic candidates (de Melo Pereira, de Oliveira Coelho, Magalhães Júnior, Thomaz-Soccol, & Soccol, 2018).

Strains capable of producing exopolysaccharides (EPS) are more resistant and therefore more suitable for industrial applications, as the production of these polymers seems to be precisely related to the response to stress. In an *Lb. paracasei* strain, the heterologous expression of ß-glucans significantly increased tolerance to heat, gastric acidity, and bile (Stack, Kearney, Stanton, Fitzgerald, & Ross, 2010). Hence, the selection of EPS-producing strains, which can also be made on the basis of the presence in the genome of specific sequences coding for the synthesis of EPS, can pave the way to the production of functional foods with improved sensory characteristics and increased cell viability during processing, storage, and GIT transit. Moreover, EPS-producing probiotics can also have a prebiotic potential, as EPS can improve the growth of other beneficial microorganisms in the intestine (Russo et al., 2012). If possible, process conditions can be modified to optimize microbial viability. In the processing of semihard Canestrato Pugliese cheese with the addition of bifidobacteria, the traditional cheesemaking process was modified, especially the heating time of the curd in hot whey was reduced to limit acidification by the starters and consequently improving the survival of probiotics during the process (Corbo, Albenzio, De Angelis, Sevi, & Gobbetti, 2001).

A possible biotechnological strategy is the selection of genetically more robust strains against given stress. Indeed, prolonged exposure of microbial cells to stress can induce spontaneous selection of more resistant mutants. Prolonged exposure to pH 2 made it possible to obtain acid-resistant phenotypes in bifidobacteria. The mutants were also more resistant to NaCl (6%–10%) and heat treatments (60°C–70°C for 10 min) and showed greater fermentation power and enzymatic activity (Collado & Sanz, 2007). The application of adequate-selective pressures can induce an improvement both in stress tolerance and in health-related functional properties, such as adhesion to enterocytes and pathogen competitive exclusion (Du Toit, Vesterlund, Gueimonde, & Salminen, 2013).

In the case of functional foods containing only probiotic microorganisms without starters, it is necessary to ensure that the former can grow in the food. Probiotics are known to have a poor ability to grow in milk; therefore, it is difficult to obtain high viability in fermented milk since probiotics have low ß-galactosidase activity. The use of ultrasound has proven to offer some advantages in this context. Low-intensity treatments can promote the activity of ß-galactosidase, which results in an increase in growth and a decrease in the lag phase in lactobacilli and bifidobacteria (Gholamhosseinpour & Hashemi, 2019). In the case of bifidobacteria, an increased ability to produce oligosaccharides and a limited amount of acetic and propionic acids has also been observed, leading to undeniable advantages over the sensory characteristics of fermented milk (Nguyen, Lee, & Zhou, 2012). A similar effect has also been observed in soymilk, where the application of ultrasound at 100 W for 2–3 min has increased the growth of bifidobacteria and lactobacilli (Yeo & Liong, 2011). Ultrasound not only has positive effects on the viability of probiotics but also on their functional activities. An increase in antioxidant activity

and ß-glucosidase activity has been observed in *Lb. plantarum*, which transforms isoflavone glucosides into aglycones, powerful bioactive molecules (Gholamhosseinpour & Hashemi, 2019).

In the case of the already discussed competition issues of probiotics with starters, which can occur especially in fermented milk products, it is possible to intervene in different ways. Obviously, the most immediate approach involves the increase of the concentration of the probiotic culture respect to the starter, which can favor the survival of probiotics since the acidification by the starter is slower, and the probiotics are in a favorable pH range for longer time. In the case of doogh, a fermented milk, the inoculation of the probiotics *Lb. acidophilus* and *B. lactis* four- or eightfold compared to standard inoculation resulted in the highest viability at the end of fermentation as well as at early days of refrigerated storage (Roudsari, Sohrabvandi, Homayouni Rad, & Mortazavian, 2013). However, an excessively high inoculation of probiotics can negatively affect the sensory profile of the product. High inoculations of *Lb. acidophilus* in Minas cheese resulted in high counts during storage but also in lower pH and higher amounts of organic acids, with a negative effect on aroma, taste, and texture and a reduced overall acceptability (Gomes et al., 2011). Therefore, the use of high probiotic counts should be carefully evaluated by taking into consideration both technological and sensory aspects.

Since starters grow rapidly during fermentation and can affect probiotics' growth by producing inhibiting substances, inoculating the starters later than probiotics can help maintain their viability. In "two-stage fermentation", the initial phase of fermentation is carried out by probiotics and is then completed by the starters. This procedure, which allows probiotics to reach the end of the lag phase or the beginning of the log phase, causes their viability to be higher at the end of fermentation, even if this can last longer. Such fermentation can also be carried out through a joint inoculation of starters and probiotics in the mixture. The fermentation mix is initially incubated at 37°C to enable the development of probiotics and subsequently at a higher temperature to allow the development of the starter and the formation of the desired sensory characteristics (Shafiee et al., 2010).

Another approach for improving the compatibility of probiotics with starters is based on the reduction or complete removal of *Lb. delbrueckii* subsp. *bulgaricus*, as this microorganism in yoghurt is responsible for the phenomenon of postacidification and the production of hydrogen peroxide, which can inhibit probiotics (Güler-Akin & Akin, 2007). However, this microorganism contributes greatly to the aroma of yoghurt, both through its proteolytic activity and by producing acetaldehyde and organic acids; therefore, its elimination may compromise the sensory characteristics of the product (Hao et al., 2011). To improve the survival of oxygen-sensitive probiotics in yoghurt and fermented milk, a selection of appropriate combinations of probiotics and oxygen-consuming *Strept. thermophilus* strains is also suggested. *Strept. thermophilus* requires a large amount of dissolved oxygen for its optimal functioning, which is made possible by mixing and stirring treatments during the homogenization, cooling, and inoculation of milk. The growth of *Strept. thermophilus* during fermentation consumes a large amount of oxygen, which contributes to promoting the survival of anaerobic probiotics (Talwalkar & Kailasapathy, 2004).

4.2 Strain cultivation

An alternative strategy that can be effective in improving the ability of probiotics to survive in food during production, storage, and GIT transit is based on adaptation to stress and cross-protection responses. As previously discussed, a wide range of environmental conditions can be stressful for probiotics and consequently cause their injury or death. However, when microorganisms are exposed to sublethal levels of specific stress, a defense mechanism against intracellular damage may be activated. This phenomenon is also known in pathogens. Indeed, *Listeria monocytogenes* can tolerate lethal pH values when pregrown at suboptimal acid conditions (Gahan, O'Driscoll, & Hill, 1996). This event, which is undesirable in pathogenic microorganisms, can become a crucial point in the development of functional foods containing a large number of probiotic microorganisms resistant to processing, storage, and GIT transit. Indeed, through this strategy, a significant increase in the tolerance of probiotics toward acid stress, oxygen, temperature, osmotic pressure, and bile can be obtained (Table 28.1).

When microorganisms are exposed to environmental stress, a cell can activate two different defense mechanisms. The first involves cellular repair processes, morphological modifications, and production of molecules whose intracellular accumulation results in an increase of tolerance to a higher level of the same stress (homologous stress). *Lb. rhamnosus* exposed for 1 h to pH 4 significantly increased its tolerance to a 2-h treatment at pH 2.5, with a recovery of viability of about 4 log CFU/mL compared to the non-pretreated cells. Similarly, a strain of *Lb. reuteri* pretreated at pH 3.5 tolerated a better exposure to pH 2.5, with an increase in its viability of more than 2 log compared to control cells (Saarela et al., 2004). Exposure to suboptimal pH levels pushes the cells to activate defense mechanisms against acidity, such as the maintenance of transmembrane ΔpH homeostasis, the modification of the composition of the cell membrane, as well as the protection and restoration of DNA and proteins (Kajfasz & Quivey, 2011). The onset of homologous resistance to different stresses (NaCl, temperature, oxygen, etc.) has been observed in many probiotic microorganisms (Du Toit et al., 2013).

The second mechanism is linked to the appearance of heterologous stress resistance. Practically, tolerance to a specific stress can be increased following exposure to a different type of stress. In several *Lactobacillus* species, exposure to bile

TABLE 28.1 Most relevant effects of cross adaptation to stress reported in probiotics.

Microorganism	Preadaptation condition	Beneficial effect	References
Lb. paracasei	0.3 M NaCl, 30 min	Enhanced protection during spray-drying	Desmond et al. (2002)
B. animalis, B. longum	pH 3.5, 1 h 47°C, 1 h	Enhanced tolerance to 1.4% bile	Saarela et al. (2004)
B. longum, B. catenulatum	pH 2, 16 h	Enhanced tolerance to 6%–10% NaCl, 1%–3% bile, and temperature (60°C–70°C)	Collado and Sanz (2007)
Lb. plantarum	45°C, pH 6.5, 30 min	Enhanced heat resistance, enhanced stability during storage at 37°C	Liao et al. (2010)
B. lactis	Heat shock at 60°C, 10 min	Increased adhesiveness to human colonic mucus	Du Toit et al. (2013)
Lc. lactis subsp. *lactis*	42°C, 15 min	Enhanced tolerance to H_2O_2, acid, and ethanol; morphological modifications	Kang, Jeon, Shin, Kwon, and So (2015)
Lb. acidophilus	Growth in different MRS and GEM media (starvation)	Enhanced tolerance to freeze-drying and extrusion encapsulation; cell elongation	Senz, van Lengerich, Bader, and Stahl (2015)
Lb. rhamnosus	Precultivation at 2%–4% NaCl, pH 4.5–6.5	Increased survival in yoghurt during storage	Settachaimongkon et al. (2015)
B. animalis subsp. *lactis*	Precultivation at 1.5% NaCl, pH 5.0–7.0	Increased viable counts during fermentation, increased survival in yoghurt during storage	(Settachaimongkon et al., 2015)
Lb. rhamnosus	pH 2.5, or NaCl 0.6 M, 1 h	Enhanced tolerance to H_2O_2	Zhang et al. (2018)
Ent. faecium	52°C, 15 min	Enhanced survival to H_2O_2, ethanol, acid, and alkali; decrease of unsaturated membrane fatty acid ratio	Shin, Kang, Kim, and So (2019)
Lb. pentosus	Growth with 2% of vegetable edible oils (sunflower, olive, linseed, soy, corn, almond, and argan)	Improved growth rate; improved tolerance to low pH and bile	Alonso García et al. (2019)

salts leads to an increase in thermotolerance (Desmond, Stanton, Fitzgerald, Collins, & Paul Ross, 2002). More recently, it has been observed that exposure to acidity and bile increase tolerance to heat and low temperatures in *Lb. kefiranofaciens* (Chen, Tang, & Chiang, 2017). Even starvation that is the lack or depletion of nutrients in the environment can induce an increase in tolerance toward various stresses. Starved *Lc. lactis* cells were more resistant to bile salts and oxidative stress, and starvation in lactobacilli and bifidobacteria induced an increase in tolerance to low temperatures and storage, as well as to –20°C freezing (Wang, Delettre, Corrieu, & Béal, 2011). One of the main mechanisms underlying the onset of cross-resistance consists in the synthesis of different types of stress proteins, including chaperonins (GroEL and GroES) responsible for protein folding, heat shock proteins, proteins involved in DNA replication, and cold shock proteins (Gaucher et al., 2019). Another mechanism, which allows countering osmotic stress, consists in the accumulation of the so-called compatible solutes, small water-soluble organic molecules that act as osmolytes. These molecules are sugars and polyols (e.g., trehalose and glycerol) and amino acids (proline, glutamate, γ-aminobutyrate, and glycine betaine), and their role is the maintenance of the conformation of proteins subjected to osmotic constraints (Le Marrec, 2011). The production of compatible solutes or chaperonins can be amplified using genetic engineering, through modification of the homologous or heterologous expression of specific genes that regulate the production of these substances. By inducing overexpression of chaperonins in *Lb. paracasei*, an improvement in the stability of the strain was observed, and overexpression of the betaine uptake system increased tolerance to acidity and osmolarity in *Lb. salivarius* (Gueimonde & Sánchez, 2012). Although quite appealing, the use of genetic engineering, however, is still unallowed in most countries, as well as feared by a large part of consumers.

Other mechanisms involve the regulation of the composition of the different elements of the bacterial envelope, such as cell membrane, cell wall, and S-layer (Gaucher et al., 2019). In recent years, various *omic* technologies have made it possible to deepen and clarify the mechanisms of response to stress in probiotic microorganisms, giving a big boost to the industrial exploitation of these mechanisms in the context of functional foods.

Traditionally, the production of biomass from microbial cultures in optimal or suboptimal conditions is carried out in batch. However, other less conventional fermentation technologies can be used to produce more robust probiotic cultures. Continuous culture has shown to provide several benefits in terms of microbial viability. *B. longum* grown under continuous

conditions showed increased tolerance to oxidative stress, *Lc. lactis* was more resistant to freeze-drying and GIT transit, and *Lb. rhamnosus* showed a better tolerance to acid stress (Lacroix & Yildirim, 2007). Immobilized cell technology can also generate several advantages. Compared to cells cultured in batch, *B. longum* obtained in the first condition has been shown to be more resistant to freezing and exposure to oxygen, probably due to structural modifications of the membrane and cell wall. In addition, greater resistance to some antibiotics has also been observed, which is interesting in the prospect of restoring intestinal microflora in people under antibiotics (Doleyres, Fliss, & Lacroix, 2004).

The application of unconventional or suboptimal cultivation conditions can significantly improve the ability of probiotics to survive in foods at high concentrations. *Lb. rhamnosus* GG and *B. lactis* BB12 grown under conditions of high NaCl concentration and low pH survived better in set-type yoghurt than cultures grown in optimal conditions and even influenced the sensory characteristics of the product (Settachaimongkon et al., 2015). A similar effect has been observed for *Lb. acidophilus* preadapted at 4°C for 18 h or at 45°C for 15 min and added to ice cream. The adaptation conditions improved stability of *Lb. acidophilus* in ice cream, although the magnitude of improvement was small (Ergin et al., 2016). Again, *Lb. plantarum* pretreated in the presence of citric acid improved its survival in various fruit juices, such as cranberry, pomegranate, and lemon and lime juices. Furthermore, a significant increase in the expression of genes related to the synthesis of cyclopropane fatty acid and *cis*-11,12-methylene octadecanoic acid has been observed, which may have caused a decrease in the fluidity and permeability of the membrane, thus preventing the cells from proton influx during storage in low pH fruit juices (Srisukchayakul, Charalampopoulos, & Karatzas, 2018). Furthermore, the effect of sublethal conditions (temperature, acid pH, and presence of hydrogen peroxide) on the survival to drying, storage, and GIT transit of *Pc. acidilactici* and *Lb. plantarum* in spray-dried orange juice was studied. Sublethal treatments did not affect drying stability and GIT transit, while strain survival was higher during 180-day storage (Barbosa, Borges, & Teixeira, 2015).

4.3 Addition of protective ingredients

The exogenous addition of protective substances is the most direct way to protect the viability of probiotics from the stresses accumulated in the food matrix, during the production process and the GIT transit. The compounds most frequently used to this purpose are carbohydrates, whose protective capacity can be explained based on two effects. The first of these concerns their ability to form glassy states that embed microbial cells, limiting the chemical and physical modifications that can put their viability at risk. Furthermore, sugars, especially those with low molecular weight, interact with the polar heads in the cell membranes, replacing the water molecules and stabilizing the membranes themselves during technological stresses. Low-molecular-weight sugars, such as trehalose, sucrose, and lactulose, can improve the survival of probiotics during freezing, dehydration, and storage both in products of animal and plant origin (Betoret, Calabuig-Jiménez, Patrignani, Lanciotti, & Dalla Rosa, 2017). Besides, they have also shown to have direct functional activities, such as a bifidogenic effect and an increase in the hydrophobicity of the surface of probiotic cells, which is related to the ability to adhere to and interact with the intestinal wall (Dlamini, Langa, Aiyegoro, & Okoh, 2019).

Even the most complex polysaccharides and fibers can improve the viability of probiotics in food. These compounds belong to the category of prebiotics, whose activity is typically functional. In fact, these are diet compounds that reach the gut in an undigested form and are selectively fermented by the intestinal microbiota, thus stimulating their growth and activity. However, their stimulation activity also takes place toward probiotics added to foods. Consequently, the development of functional foods that contain both probiotics and prebiotics (symbiotic foods) can provide a double physiological advantage. Typically, prebiotic substances mainly come from plant food sources. In fact, inulin and fructooligosaccharides (FOS), widely used for this purpose, are commonly extracted from onions, bananas, wheat, artichokes, garlic, and other whole foods. Other widely used oligosaccharides, such as galactooligosaccharides (GOS) and human milk oligosaccharides, are of animal origin (Anadón, Martínez-Larrañaga, Arés, & Martínez, 2016). Even microorganisms are capable of producing extracellular substances that act as prebiotics, such as EPS (İspirli, Demirbaş, & Dertli, 2018). The effectiveness of prebiotics in the context of functional probiotic foods is linked to the fact that these substances can be metabolized and used as a source of energy by probiotics. Furthermore, owing to their physicochemical characteristics, they can effectively protect cell envelopes, increase the glass transition temperatures in the aqueous phase, retain water, and prevent the formation of ice crystals that may cause mechanical damage to the cells themselves (Tymczyszyn, Gerbino, Illanes, & Gómez-Zavaglia, 2011). Due to these activities, the effects essentially consist in protecting probiotics against acidity, high temperatures, and dehydration. Their addition to food results in an improvement in the stability of probiotics during processing, storage, and *in vitro* digestion. In blended carrot and orange juices, the presence of 2% inulin improved the survival of *Lb. plantarum* during storage for 30 days at 4°C and during *in vitro* digestion, and a mixture of inulin, FOS, and GOS had a similar effect on *Lb. casei* in a blended red fruit beverage (Bernal-Castro, Díaz-Moreno, & Gutiérrez-Cortés, 2019; Valero-Cases & Frutos, 2017).

Several other substances can be used to improve the viability of probiotics in food, such as vitamins, minerals, dairy protein extracts (both caseins and whey proteins), hydrocolloids (gelatin, Arabic gum), polyalcohols, and yeast extract. These

supplements improve the growth capacity of probiotics in food matrices, as well as tolerance to storage, as they firstly reduce the redox potential, thus facilitating the survival of anaerobic strains, and secondly increase the buffer capacity of the medium, resulting in a relief of acid stress.

Microalgae can also make an important contribution to the viability of probiotics in food. These organisms are particularly rich in amino acids, polyunsaturated fatty acids, carbohydrates, minerals, and vitamins, which have various direct functional effects, such as antioxidant, antiinflammatory, and hepatoprotective activity. Thanks to their composition they can improve the growth of probiotics in foods, including cheeses, yoghurt, and fermented soy drinks (Patel et al., 2019).

An innovative approach in this context is the use of by-products, mainly of plant origin, of the food industry as promoters of the tolerance of probiotics to stress. The enhancement of said by-products even has a valuable environmental impact, as it partially solves industrial waste issues. The by-products of different fruits, such as passion-fruit, guava, and orange, enhanced the resistance of a probiotic *Lb. casei* strain to simulated GIT conditions in probiotic fermented goat milk and cereal-based fermented products, also improving the functional characteristics of the products, for example, by stimulating the production of folate by probiotics (Casarotti, Borgonovi, Batista, & Penna, 2018). Again, the survival of *Lb. salivarius* and *Lb. plantarum* to freeze-drying and cold storage was stimulated by the presence of malt sprout (a by-product from the malt industry), and a similar result in the case of spray-drying was observed in *Lb. plantarum* in the presence of whole and defatted okara, a by-product from soymilk manufacturing (Cejas et al., 2017).

4.4 Encapsulation

Traditionally, probiotic microorganisms have been added to foods as free cells. However, to improve the probiotics' ability to remain viable long enough to ensure beneficial effects, a favorable solution is offered by encapsulation. It consists of a technique in which a sensitive component (in this case probiotic cells) is segregated from the surrounding environment as it is enclosed within a proper substance that will release it at the target site. The encapsulation process leads to the formation of a core, made of a matrix in which the cells are dispersed to ensure an active metabolism for a long time, and an inert shell or wall material acting as a barrier against the external stresses. Some benefits of probiotics' encapsulation include the controlled release of cells in the target site (i.e., colon), the protection from bacteriophages, and detrimental factors, leading to an increase in survival during processing and storage, and its ease of use (Table 28.2) (Martín, Lara-Villoslada, Ruiz, & Morales, 2015; Rathore, Desai, Liew, Chan, & Heng, 2013).

TABLE 28.2 Applications of encapsulation to deliver probiotics into foods.

Encapsulation material	Encapsulation technology	Microorganism	Food	Beneficial effect	References
Alginate	Droplet extrusion/ emulsion	*B. longum*	Cheddar cheese	Protection during storage and GIT transit	Amine et al. (2014)
Alginate	Extrusion	*Lb. reuteri*	Dry fermented sausages	Protection during drying	Muthukumarasamy and Holley (2006)
Alginate + human like collagen	Electrostatic droplet generation	*B. longum*	Skim milk	Protection during 4°C storage	Su et al. (2011)
Alginate + resistant starch	Emulsion	*Lb. casei, B. lactis*	Ice cream	Protection during storage at −20°C	Homayouni et al. (2008a)
Whey proteins + alginate	Droplet extrusion + electrostatic deposition	*Lb. rhamnosus*	Fruit juice	Protection during GIT transit	Doherty et al. (2012)
Whey proteins/whey proteins + resistant starch	Spray drying	*Lb. rhamnosus*	Apple juice	Protection during storage at 25°C	Ying et al. (2013)
Legume protein isolates + alginate	Extrusion	*B. adolescentis*	Yoghurt	Protection during storage and GIT transit	Khan et al. (2013)
Monoglycerides + oil/anhydrous milk fat	Emulsion	*Lb. rhamnosus*	Ice cream	Protection during cold storage	Calligaris et al. (2018), Marino et al. (2017)

The material used and the technology applied depend on several factors such as the food process, product features, and storage time and conditions. Several materials have been used to deliver probiotic bacteria. Among polysaccharides, alginate is the most widely used since it is inexpensive, nontoxic, and biocompatible. However, the porosity of the resulting capsules and the sensitivity to acid conditions reduce the applicability of this material alone (Gouin, 2004). These issues can be addressed by coating the capsules as well as by mixing alginate with other polymers such as whey proteins, gums, gelatin, pectin, carrageenan, and starch (Calinoiu, Ştefanescu, Pop, Muntean, & Vodnar, 2019; Doherty et al., 2012). Differently from alginate, gellan gum and xanthan gum mixtures are highly resistant to acid condition, while cellulose acetate phthalate and carboxymethyl cellulose provide good cell protection during the GIT transit (Fávaro-Trindade & Grosso, 2002). k-Carrageenan beads ensure probiotic viability, but the resulting gels are brittle and unable to withstand stresses (Burgain, Gaiani, Linder, & Scher, 2011). A promising material is represented by starch, especially the resistant starch (Himaize). The latter is not digested by pancreatic enzymes and can reach the colon where it will be fermented by the resident microbiota. This feature allows the release of bacterial cells in the large intestine and at the same time is responsible for the prebiotic functionality. Moreover, starch granules are suitable for probiotic adherence, which promotes the delivery of cells in a viable and metabolically active state (Burgain et al., 2011).

Among proteins, milk proteins are natural vehicles of probiotics and are widely used due to their excellent physicochemical characteristics, such as the gelation properties (Gomez-Mascaraque, Morfin, Pérez-Masiá, Sanchez, & Lopez-Rubio, 2016; Melchior, Marino, Innocente, Calligaris, & Nicoli, 2020) However, in recent years, plant proteins (from pea, chickpea, lentil, and broad bean) have also been exploited showing promising ability in protecting probiotic cells during storage and GIT transit (Khan, Korber, Low, & Nickerson, 2013).

While protein and polysaccharide materials have been wieldy studied to deliver probiotic bacteria, little is known about lipids. *B. longum* was immobilized in a cocoa butter-based matrix resulting in increased viability of the cells in a model beverage during storage (Lahtinen, Ouwehand, Salminen, Forssell, & Myllärinen, 2007). Solid lipid microparticles represented a good carrier for *B. lactis* and *Lb. acidophilus*, which were protected against low temperature and GIT transit (de Lara Pedroso, Thomazini, Heinemann, & Favaro-Trindade, 2012). More recently, monoglyceride structured emulsions containing oil or anhydrous milk fat as lipid phase were used to protect *Lb. rhamnosus* during cold storage as well as in ice cream during processing, storage, and GIT transit (Calligaris, Marino, Maifreni, & Innocente, 2018; Melchior et al., 2021; Marino et al., 2017).

Among the technologies applied, extrusion is the oldest and most common one to encapsulate probiotics. It consists in extruding hydrocolloid solutions containing cells through a nozzle at high pressure. This technique is cheap, simple and allows to maintain cells in a viable state due to the mild process conditions adopted, but it is difficult to use in large-scale production due to the low throughput (Burgain et al., 2011). Spray drying is more suitable for industrial application, and it is based on the atomization of cells suspended in a polymeric solution into hot drying air, resulting in rapid water evaporation. The negative side of this process is represented by the high temperature used, which may reduce the probiotic viability due to simultaneous dehydration, thermal, and oxygen stresses imposed to bacteria. To overcome this problem, protectants, such as soluble fiber or trehalose, can be added to the suspension before drying (Semyonov et al., 2010). More recently, other strategies have been proposed such as the spray freeze-drying method. During this process, the probiotic suspension is atomized into a cold vapor phase leading to the formation of frozen droplets that are then dried in freeze conditions (de Vos, Faas, Spasojevic, & Sikkema, 2010). Alternatives to conventional spray drying are two-step drying, based on the combination of spray drying and vacuum drying at mild temperatures, spray chilling, and ultrasonic vacuum spray drying, which decrease the thermal and oxidative stresses due to the use of an ultrasonic nozzle combined with low temperatures and vacuum atmosphere during the process (Martín et al., 2015). Emulsification can also be used to encapsulate probiotic cells. It involves the homogenization of a dispersion composed by a cell-polymer solution (dispersed phase) in oil (continuous phase). Then, the dispersed phase must be cross-linked within the continuous phase to form gel particles sized from 0.2 to 5000 μm. This technique enhances the protection of lactobacilli and bifidobacteria against the process, storage, and GIT adverse conditions (Chitprasert, Sudsai, & Rodklongtan, 2012; Homayouni, Ehsani, Azizi, Razavi, & Yarmand, 2008b). More recently, novel emulsion-based strategies have been exploited such as double or multiple emulsions or the application of emulsion as a food ingredient (Marino et al., 2017). Finally, more innovative methods are represented by electrohydrodynamic techniques, namely, electrospinning and electrospraying, which allow the formation of fiber or capsules having a size of few nanometers. These strategies have been proven to enhance the viability of *Lb. plantarum* and *B. animalis* subsp. *lactis* during storage at relatively high humidity when encapsulated in whey protein matrices (Gomez-Mascaraque et al., 2016).

5 Final considerations

In recent decades, the interest of the scientific world concerning the health benefits induced by probiotic microorganisms has increased considerably, and consequently, many products have appeared on the market aimed at meeting the ever-growing interest of consumers. When claiming the health benefits, the food industry must ensure that microbial viability remains

sufficiently high until it reaches the target site. The most interesting and innovative approaches to achieve this goal seem to be (1) an accurate selection of the strains and their functional characteristics, using both conventional strategies and an *omic* approach, (2) the application of suboptimal growth conditions of probiotics in biomass cultivation phase, where the cellular mechanisms of adaptation and the related molecular markers must be understood to quickly identify the most performing strains and the most suitable growth conditions, (3) the use of prebiotics, which can help in maintaining the viability of probiotics in the food while stimulating the performance of the intestinal microbiota, (4) the exploitation of specific by-products from the agro-industry, which can stabilize probiotics and improve the environmental impact of specific supply chains, and (5) the identification of new polymeric matrices and new encapsulation technologies to optimize the microbial survival in the various stressful conditions encountered during food processing and GIT transit.

In this regard, there is a need for a constant supply of knowledge on the characteristics of the microbial strains, on their ability to interact with the product during the processing and on the host-microbe interactions in the GIT, also supported by appropriate clinical trials aimed at accurately quantifying the physiological effects resulting from the consumption of probiotic foods. These data will elucidate the appropriateness of foods containing probiotic microorganisms in the human diet and legitimize their inclusion in nutritional guidelines, also in consideration of market demands.

References

Ahn, J. B., Hwang, H. J., & Park, J. H. (2001). Physiological responses of oxygen-tolerant anaerobic *Bifidobacterium longum* under oxygen. *Journal of Microbiology and Biotechnology*, *11*, 443–451.

Alonso García, E., Pérez Montoro, B., Benomar, N., Castillo-Gutiérrez, S., Estudillo-Martínez, M. D., Knapp, C. W., et al. (2019). New insights into the molecular effects and probiotic properties of *Lactobacillus pentosus* pre-adapted to edible oils. *LWT-Food Science and Technology*, *109*, 153–162.

Amine, K. M., Champagne, C. P., Raymond, Y., St-Gelais, D., Britten, M., Fustier, P., et al. (2014). Survival of microencapsulated *Bifidobacterium longum* in Cheddar cheese during production and storage. *Food Control*, *37*, 193–199.

Anadón, A., Martínez-Larrañaga, M. R., Arés, I., & Martínez, M. A. (2016). Prebiotics and probiotics: An assessment of their safety and health benefits. In R. R. Watson, & V. R. Preedy (Eds.), *Probiotics, prebiotics, and synbiotics: Bioactive foods in health promotion* (pp. 3–23). London, UK: Academic Press Inc.

Anandharaj, M., Rani, R. P., & Swain, M. R. (2018). Production of high-quality probiotics by fermentation. In V. K. Gupta, H. Treichel, V. Shapaval, L. A. de Oliveira, & M. G. Tuohy (Eds.), *Microbial functional foods and nutraceuticals* (pp. 235–266). Hoboken, NJ: Wiley.

Barbosa, J., Borges, S., & Teixeira, P. (2015). Influence of sub-lethal stresses on the survival of lactic acid bacteria after spray-drying in orange juice. *Food Microbiology*, *52*, 77–83.

Barros, C. P., Guimarães, J. T., Esmerino, E. A., Duarte, M. C. K. H., Silva, M. C., Silva, R., et al. (2020). Paraprobiotics and postbiotics: Concepts and potential applications in dairy products. *Current Opinion in Food Science*, *32*, 1–8.

Bernal-Castro, C. A., Díaz-Moreno, C., & Gutiérrez-Cortés, C. (2019). Inclusion of prebiotics on the viability of a commercial *Lactobacillus casei* subsp. *rhamnosus* culture in a tropical fruit beverage. *Journal of Food Science and Technology*, *56*, 987–994.

Betoret, E., Calabuig-Jiménez, L., Patrignani, F., Lanciotti, R., & Dalla Rosa, M. (2017). Effect of high pressure processing and trehalose addition on functional properties of mandarin juice enriched with probiotic microorganisms. *LWT-Food Science and Technology*, *85*, 418–422.

Bigliardi, B., & Galati, F. (2013). Innovation trends in the food industry: The case of functional foods. *Trends in Food Science and Technology*, *31*, 118–129.

Burgain, J., Gaiani, C., Linder, M., & Scher, J. (2011). Encapsulation of probiotic living cells: From laboratory scale to industrial applications. *Journal of Food Engineering*, *104*, 467–483.

Calinoiu, L. F., Ştefanescu, B. E., Pop, I. D., Muntean, L., & Vodnar, D. C. (2019). Chitosan coating applications in probiotic microencapsulation. *Coatings*, *9*, 1–21.

Calligaris, S., Marino, M., Maifreni, M., & Innocente, N. (2018). Potential application of monoglyceride structured emulsions as delivery systems of probiotic bacteria in reduced saturated fat ice cream. *LWT-Food Science and Technology*, *96*, 329–334.

Casarotti, S. N., Borgonovi, T. F., Batista, C. L. F. M., & Penna, A. L. B. (2018). Guava, orange and passion fruit by-products: Characterization and its impacts on kinetics of acidification and properties of probiotic fermented products. *LWT-Food Science and Technology*, *98*, 69–76.

Cavallini, D. C. U., Manzoni, M. S. J., Bedani, R., Roselino, M. N., Celiberto, L. S., Vendramini, R. C., et al. (2016). Probiotic soy product supplemented with isoflavones improves the lipid profile of moderately hypercholesterolemic men: A randomized controlled trial. *Nutrients*, *8*, 52.

Cejas, L., Romano, N., Moretti, A., Mobili, P., Golowczyc, M., & Gómez-Zavaglia, A. (2017). Malt sprout, an underused beer by-product with promising potential for the growth and dehydration of lactobacilli strains. *Journal of Food Science and Technology*, *54*, 4464–4472.

Chen, M. J., Tang, H. Y., & Chiang, M. L. (2017). Effects of heat, cold, acid and bile salt adaptations on the stress tolerance and protein expression of kefir-isolated probiotic *Lactobacillus kefiranofaciens* M1. *Food Microbiology*, *66*, 20–27.

Chitprasert, P., Sudsai, P., & Rodklongtan, A. (2012). Aluminum carboxymethyl cellulose-rice bran microcapsules: Enhancing survival of *Lactobacillus reuteri* KUB-AC5. *Carbohydrate Polymers*, *90*, 78–86.

Collado, M. C., & Sanz, Y. (2007). Induction of acid resistance in *Bifidobacterium*: A mechanism for improving desirable traits of potentially probiotic strains. *Journal of Applied Microbiology*, *103*, 1147–1157.

Corbo, M. R., Albenzio, M., De Angelis, M., Sevi, A., & Gobbetti, M. (2001). Microbiological and biochemical properties of Canestrato pugliese hard cheese supplemented with bifidobacteria. *Journal of Dairy Science*, *84*, 551–561.

de Almeida, J., dos, S. O., Dias, C. O., Pinto, S. S., Pereira, L. C., Verruck, S., et al. (2018). Probiotic Mascarpone-type cheese: Characterisation and cell viability during storage and simulated gastrointestinal conditions. *International Journal of Dairy Technology*, *71*, 195–203.

de Lara Pedroso, D., Thomazini, M., Heinemann, R. J. B., & Favaro-Trindade, C. S. (2012). Protection of *Bifidobacterium lactis* and *Lactobacillus acidophilus* by microencapsulation using spray-chilling. *International Dairy Journal*, *26*, 127–132.

de Melo Pereira, G. V., de Oliveira Coelho, B., Magalhães Júnior, A. I., Thomaz-Soccol, V., & Soccol, C. R. (2018). How to select a probiotic? A review and update of methods and criteria. *Biotechnology Advances*, *36*, 2060–2076.

de Vos, P., Faas, M. M., Spasojevic, M., & Sikkema, J. (2010). Encapsulation for preservation of functionality and targeted delivery of bioactive food components. *International Dairy Journal*, *20*, 292–302.

De Vuyst, L. (2000). Technology aspects related to the application of functional starter cultures. *Food Technology and Biotechnology*, *38*, 105–112.

Desmond, C., Stanton, C., Fitzgerald, G. F., Collins, K., & Paul Ross, R. (2002). Environmental adaptation of probiotic lactobacilli towards improvement of performance during spray drying. *International Dairy Journal*, *12*, 183–190.

Diplock, A. T., Aggett, P. J., Ashwell, M., Bornet, F., Fern, E. B., & Roberfroid, M. B. (1999). Scientific concepts of functional foods in Europe: Consensus document. *British Journal of Nutrition*, *81*, S1–S27.

Dlamini, Z. C., Langa, R. L. S., Aiyegoro, O. A., & Okoh, A. I. (2019). Safety evaluation and colonisation abilities of four lactic acid bacteria as future probiotics. *Probiotics and Antimicrobial Proteins*, *11*, 397–402.

Doherty, S. B., Auty, M. A., Stanton, C., Ross, R. P., Fitzgerald, G. F., & Brodkorb, A. (2012). Application of whey protein micro-bead coatings for enhanced strength and probiotic protection during fruit juice storage and gastric incubation. *Journal of Microencapsulation*, *29*, 713–728.

Doleyres, Y., Fliss, I., & Lacroix, C. (2004). Increased stress tolerance of *Bifidobacterium longum* and *Lactococcus lactis* produced during continuous mixed-strain immobilized-cell fermentation. *Journal of Applied Microbiology*, *97*, 527–539.

Du Toit, E., Vesterlund, S., Gueimonde, M., & Salminen, S. (2013). Assessment of the effect of stress-tolerance acquisition on some basic characteristics of specific probiotics. *International Journal of Food Microbiology*, *165*, 51–56.

Ergin, F., Atamer, Z., Asci Arslan, A., Comak Gocer, E. M., Demir, M., Samtlebe, M., et al. (2016). Application of cold- and heat-adapted *Lactobacillus acidophilus* in the manufacture of ice cream. *International Dairy Journal*, *59*, 72–79.

Fávaro-Trindade, C. S., & Grosso, C. R. F. (2002). Microencapsulation of *L. acidophilus* (La-05) and *B. lactis* (Bb-12) and evaluation of their survival at the pH values of the stomach and in bile. *Journal of Microencapsulation*, *19*, 485–494.

Felis, G. E., Dellaglio, F., & Torriani, S. (2009). Taxonomy of probiotic microorganisms. In D. Charalampopoulos, & R. A. Rastall (Eds.), *Prebiotics and probiotics science and technology* (pp. 591–637). New York, NY: Springer.

Fernandez-Pacheco Rodríguez, P., Arévalo-Villena, M., Zaparoli Rosa, I., & Briones Pérez, A. (2018). Selection of potential non-*Saccharomyces* probiotic yeasts from food origin by a step-by-step approach. *Food Research International*, *112*, 143–151.

Finn, S., Condell, O., McClure, P., Amézquita, A., & Fanning, S. (2013). Mechanisms of survival, responses, and sources of *Salmonella* in low-moisture environments. *Frontiers in Microbiology*, *4*, 331.

Fredua-Agyeman, M., & Gaisford, S. (2015). Comparative survival of commercial probiotic formulations: Tests in biorelevant gastric fluids and real-time measurements using microcalorimetry. *Beneficial Microbes*, *6*, 141–151.

Gahan, C. G. M., O'Driscoll, B., & Hill, C. (1996). Acid adaptation of *Listeria monocytogenes* can enhance survival in acidic foods and during milk fermentation. *Applied and Environmental Microbiology*, *62*, 3128–3132.

Gaucher, F., Bonnassie, S., Rabah, H., Marchand, P., Blanc, P., Jeantet, R., et al. (2019). Adaptation of beneficial propionibacteria, lactobacilli, and bifidobacteria improves tolerance toward technological and digestive stresses. *Frontiers in Microbiology*, *10*, 841.

Gholamhosseinpour, A., & Hashemi, S. M. B. (2019). Ultrasound pretreatment of fermented milk containing probiotic *Lactobacillus plantarum* AF1: Carbohydrate metabolism and antioxidant activity. *Journal of Food Process Engineering*, *42*, e12930.

Gomes, A. A., Braga, S. P., Cruz, A. G., Cadena, R. S., Lollo, P. C. B., Carvalho, C., et al. (2011). Effect of the inoculation level of *Lactobacillus acidophilus* in probiotic cheese on the physicochemical features and sensory performance compared with commercial cheeses. *Journal of Dairy Science*, *94*, 4777–4786.

Gomez-Mascaraque, L. G., Morfin, R. C., Pérez-Masiá, R., Sanchez, G., & Lopez-Rubio, A. (2016). Optimization of electrospraying conditions for the microencapsulation of probiotics and evaluation of their resistance during storage and in-vitro digestion. *LWT-Food Science and Technology*, *69*, 438–446.

Gouin, S. (2004). Microencapsulation: Industrial appraisal of existing technologies and trends. *Trends in Food Science and Technology*, *15*, 330–347.

Gueimonde, M., & Sánchez, B. (2012). Enhancing probiotic stability in industrial processes. *Microbial Ecology in Health & Disease*, *23*, 18562.

Güler-Akin, M. B., & Akin, M. S. (2007). Effects of cysteine and different incubation temperatures on the microflora, chemical composition and sensory characteristics of bio-yogurt made from goat's milk. *Food Chemistry*, *100*, 788–793.

Hajduk, G., Kos, B., Šušković, J., Frece, J., Pavunc, A. L., & Beganović, J. (2009). Probiotic properties of *Bifidobacterium animalis* subsp. *lactis* BB-12 in cereal flakes enriched with inulin. *Italian Journal of Food Science*, *21*, 473–486.

Hao, P., Zheng, H., Yu, Y., Ding, G., Gu, W., Chen, S., et al. (2011). Complete sequencing and pan-genomic analysis of *Lactobacillus delbrueckii* subsp. *bulgaricus* reveal its genetic basis for industrial yogurt production. *PLoS One*, *6*, e15964.

Hernández-Barrueta, T., Martínez-Bustos, F., Castaño-Tostado, E., Lee, Y., Miller, M. J., & Amaya-Llano, S. L. (2020). Encapsulation of probiotics in whey protein isolate and modified huauzontle's starch: An approach to avoid fermentation and stabilize polyphenol compounds in a ready-to-drink probiotic green tea. *LWT-Food Science and Technology*, *124*, 109131.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., et al. (2014). The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology and Hepatology*, *11*, 506–514.

Homayouni, A., Azizi, A., Ehsani, M. R., Yarmand, M. S., & Razavi, S. H. (2008a). Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. *Food Chemistry*, *111*, 50–55.

Homayouni, A., Ehsani, M. R., Azizi, A., Razavi, S. H., & Yarmand, M. S. (2008b). Growth and survival of some probiotic strains in simulated ice cream conditions. *Journal of Applied Sciences*, *8*, 379–382.

Hotchkiss, J. H., Werner, B. G., & Lee, E. Y. C. (2006). Addition of carbon dioxide to dairy products to improve quality: A comprehensive review. *Comprehensive Reviews in Food Science and Food Safety*, *5*, 158–168.

Innocente, N., Biasutti, M., Rita, F., Brichese, R., Comi, G., & Iacumin, L. (2016). Effect of indigenous *Lactobacillus rhamnosus* isolated from bovine milk on microbiological characteristics and aromatic profile of traditional yogurt. *LWT-Food Science and Technology*, *66*, 158–164.

İspirli, H., Demirbaş, F., & Dertli, E. (2018). Glucan type exopolysaccharide (EPS) shows prebiotic effect and reduces syneresis in chocolate pudding. *Journal of Food Science and Technology*, *55*, 3821–3826.

Jayamanne, V. S., & Adams, M. R. (2006). Determination of survival, identity and stress resistance of probiotic bifidobacteria in bio-yoghurts. *Letters in Applied Microbiology*, *42*, 189–194.

Kailasapathy, K. (2006). Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt. *LWT-Food Science and Technology*, *39*, 1221–1227.

Kajfasz, J. K., & Quivey, R. G. (2011). Responses of lactic acid bacteria to acid stress. In E. Tsakalidou, & K. Papadimitriou (Eds.), *Stress responses of lactic acid bacteria* (pp. 23–53). New York, NY: Springer.

Kang, C. H., Jeon, H., Shin, Y. J., Kwon, Y. J., & So, J. S. (2015). Heat adaptation improves viability of *Lactococcus lactis* subsp. *lactis* HE-1 after heat stress. *Food Science and Biotechnology*, *24*, 1823–1827.

Khan, M. I., Arshad, M. S., Anjum, F. M., Sameen, A., Aneeq-ur, R., & Gill, W. T. (2011). Meat as a functional food with special reference to probiotic sausages. *Food Research International*, *44*, 3125–3133.

Khan, M. I., Korber, D. R., Low, N. H., & Nickerson, M. T. (2013). Development of extrusion-based legume protein isolate-alginate capsules for the protection and delivery of the acid sensitive probiotic, *Bifidobacterium adolescentis*. *Food Research International*, *54*, 730–737.

Klu, Y. A. K., Phillips, R. D., & Chen, J. (2014). Survival of four commercial probiotic mixtures in full fat and reduced fat peanut butter. *Food Microbiology*, *44*, 34–40.

Klu, Y. A. K., Williams, J. H., Phillips, R. D., & Chen, J. (2012). Survival of *Lactobacillus rhamnosus* GG as influenced by storage conditions and product matrixes. *Journal of Food Science*, *77*, M659–M663.

Koebnick, C., Wagner, I., Leitzmann, P., Stern, U., & Zunft, H. J. F. (2003). Probiotic beverage containing *Lactobacillus casei* Shirota improves gastrointestinal symptoms in patients with chronic constipation. *Canadian Journal of Gastroenterology*, *17*, 655–659.

Kumpu, M., Kekkonen, R. A., Korpela, R., Tynkkynen, S., Järvenpää, S., Kautiainen, H., et al. (2015). Effect of live and inactivated *Lactobacillus rhamnosus* GG on experimentally induced rhinovirus colds: Randomised, double blind, placebo-controlled pilot trial. *Beneficial Microbes*, *6*, 631–639.

Lacroix, C., & Yildirim, S. (2007). Fermentation technologies for the production of probiotics with high viability and functionality. *Current Opinion in Biotechnology*, *18*, 176–183.

Ladero, V., & Sánchez, B. (2017). Molecular and technological insights into the aerotolerance of anaerobic probiotics: Examples from bifidobacteria. *Current Opinion in Food Science*, *14*, 110–115.

Lahtinen, S. J. J., Ouwehand, A. C. C., Salminen, S. J. J., Forssell, P., & Myllärinen, P. (2007). Effect of starch- and lipid-based encapsulation on the culturability of two *Bifidobacterium longum* strains. *Letters in Applied Microbiology*, *44*, 500–505.

Le Marrec, C. (2011). Responses of lactic acid bacteria to osmotic stress. In E. Tsakalidou, & K. Papadimitriou (Eds.), *Stress responses of lactic acid bacteria* (pp. 67–90). New York, NY: Springer.

Lee, Y. K., Margolles, A., Mayo, B., Ruas-Madiedo, P., Gueimonde, M., de los Reyes-Gavilán, C., et al. (2008). Probiotic microorganisms. In Y. K. Lee, & S. Salminen (Eds.), *Handbook of probiotics and prebiotics* (2nd ed., pp. 2–176). Hoboken, NJ: John Wiley.

Liao, Q., Hang, X., Liu, X., Pan, J., Zhang, H., & Yang, H. (2010). The influence of pH on heat stress response by probiotic *Lactobacillus plantarum* LP-Onlly. *Annals of Microbiology*, *60*, 341–348.

Mahantesha, T., Reddy, K. M. P., Kumar, N. H. P., Nara, A., Ashwin, D., & Buddiga, V. (2015). Comparative study of probiotic ice cream and probiotic drink on salivary *Streptococcus mutans* levels in 6-12 years age group children. *Journal of International Oral Health*, *7*, 47–50.

Majeed, M., Majeed, S., Nagabhushanam, K., Natarajan, S., Sivakumar, A., & Ali, F. (2016). Evaluation of the stability of *Bacillus coagulans* MTCC 5856 during processing and storage of functional foods. *International Journal of Food Science and Technology*, *51*, 894–901.

Månberger, A., Verbrugghe, P., GuÐmundsdóttir, E. E., Santesson, S., Nilsson, A., HreggviÐsson, G. Ó., et al. (2020). Taxogenomic assessment and genomic characterisation of *Weissella cibaria* strain 92 able to metabolise oligosaccharides derived from dietary fibres. *Scientific Reports*, *10*, 5853.

Marino, M., Innocente, N., Calligaris, S., Maifreni, M., Marangone, A., & Nicoli, M. C. C. (2017). Viability of probiotic *Lactobacillus rhamnosus* in structured emulsions containing saturated monoglycerides. *Journal of Functional Foods*, *35*, 51–59.

Martín, M. J., Lara-Villoslada, F., Ruiz, M. A., & Morales, M. E. (2015). Microencapsulation of bacteria: A review of different technologies and their impact on the probiotic effects. *Innovative Food Science and Emerging Technologies*, *27*, 15–25.

Melchior, S., Marino, M., Innocente, N., Calligaris, S., & Nicoli, M. C. (2020). Effect of different biopolymer-based structured systems on the survival of probiotic strains during storage and *in vitro* digestion. *Journal of the Science of Food and Agriculture*, *100*, 3902–3909.

Melchior, S., Marino, M., D'Este, F., Innocente, N., Nicoli, M. C., & Calligaris, S. (2021). Effect of the formulation and structure of monoglyceride-based gels on the viability of probiotic *Lactobacillus rhamnosus* upon *in vitro* digestion. *Food & Function*. In press.

Metchnikoff, E. (1908). *Prolongation of life, optimistic studies*. London: Butterworth-Heinemann.

Miller, C. W., Nguyen, M. H., Rooney, M., & Kailasapathy, K. (2003). The control of dissolved oxygen content in probiotic yoghurts by alternative packaging materials. *Packaging Technology and Science*, *16*, 61–67.

Mitsou, E. K., Panopoulou, N., Turunen, K., Spiliotis, V., & Kyriacou, A. (2010). Prebiotic potential of barley derived β-glucan at low intake levels: A randomised, double-blinded, placebo-controlled clinical study. *Food Research International*, *43*, 1086–1092.

Mortazavian, A. M., Ehsani, M. R., Mousavi, S. M., Reinheimer, J. A., Emamdjomeh, Z., Sohrabvandi, S., et al. (2006). Preliminary investigation of the combined effect of heat treatment and incubation temperature on the viability of the probiotic micro-organisms in freshly made yogurt. *International Journal of Dairy Technology*, *59*, 8–11.

Mortazavian, A. M., Ehsani, M. R., Mousavi, S. M., Rezaei, K., Sohrabvandi, S., & Reinheimer, J. A. (2007). Effect of refrigerated storage temperature on the viability of probiotic micro-organisms in yogurt. *International Journal of Dairy Technology*, *60*, 123–127.

Moslehi-Jenabian, S., Pedersen, L. L., & Jespersen, L. (2010). Beneficial effects of probiotic and food borne yeasts on human health. *Nutrients*, *2*, 449–473.

Muthukumarasamy, P., & Holley, R. A. (2006). Microbiological and sensory quality of dry fermented sausages containing alginate-microencapsulated *Lactobacillus reuteri*. *International Journal of Food Microbiology*, *111*, 164–169.

Nematollahi, A., Sohrabvandi, S., Mortazavian, A. M., & Jazaeri, S. (2016). Viability of probiotic bacteria and some chemical and sensory characteristics in cornelian cherry juice during cold storage. *Electronic Journal of Biotechnology*, *21*, 49–53.

Nguyen, T. M. P., Lee, Y. K., & Zhou, W. (2012). Effect of high intensity ultrasound on carbohydrate metabolism of bifidobacteria in milk fermentation. *Food Chemistry*, *130*, 866–874.

Önneby, K., Pizzul, L., Bjerketorp, J., Mahlin, D., Håkansson, S., & Wessman, P. (2013). Effects of di- and polysaccharide formulations and storage conditions on survival of freeze-dried *Sphingobium* sp.. *World Journal of Microbiology and Biotechnology*, *29*, 1399–1408.

Otieno, D. O., Ashton, J. F., & Shah, N. E. (2006). Stability of β-glucosidase activity produced by *Bifidobacterium* and *Lactobacillus* spp. in fermented soymilk during processing and storage. *Journal of Food Science*, *70*, M236–M241.

Patel, P., Jethani, H., Radha, C., Vijayendra, S. V. N., Mudliar, S. N., Sarada, R., et al. (2019). Development of a carotenoid enriched probiotic yogurt from fresh biomass of *Spirulina* and its characterization. *Journal of Food Science and Technology*, *56*, 3721–3731.

Ranadheera, C. S., Evans, C. A., Adams, M. C., & Baines, S. K. (2012). *In vitro* analysis of gastrointestinal tolerance and intestinal cell adhesion of probiotics in goat's milk ice cream and yogurt. *Food Research International*, *49*, 619–625.

Randazzo, C. L., Pitino, I., Licciardello, F., Muratore, G., & Caggia, C. (2013). Survival of *Lactobacillus rhamnosus* probiotic strains in peach jam during storage at different temperatures. *Food Science and Technology*, *33*, 652–659.

Rathore, S., Desai, P. M., Liew, C. V., Chan, L. W., & Heng, P. W. S. (2013). Microencapsulation of microbial cells. *Journal of Food Engineering*, *116*, 369–381.

Roberfroid, M. B. (2000). Defining functional foods. In G. R. Gibson, & C. M. Williams (Eds.), *Functional foods - Concept to product* (pp. 9–27). Cambridge, UK: CRC Press.

Rodríguez-Gómez, F., Romero-Gil, V., García-García, P., Arroyo-López, F. N., & Garrido-Fernández, A. (2016). Effect of green Spanish-style Manzanilla packaging conditions on the prevalence of the putative probiotic bacteria *Lactobacillus pentosus* TOMC-LAB2. *Food Science and Nutrition*, *4*, 181–197.

Roudsari, M. R., Sohrabvandi, S., Homayouni Rad, A., & Mortazavian, A. M. (2013). Combined effects of inoculation level and sequence on biochemical and microbiological characteristics of probiotic Doogh. *Iranian Journal of Pharmaceutical Research*, *12*, 299–305.

Russo, P., López, P., Capozzi, V., de Palencia, P. F., Dueñas, M. T., Spano, G., et al. (2012). Beta-glucans improve growth, viability and colonization of probiotic microorganisms. *International Journal of Molecular Sciences*, *13*, 6026–6039.

Saarela, M. (2018). Future development of probiotic dairy products. In A. Y. Tamime, & L. V. Thomas (Eds.), *Probiotic dairy products* (pp. 389–394). Hoboken, NJ: Wiley-Blackwell.

Saarela, M., Rantala, M., Hallamaa, K., Nohynek, L., Virkajärvi, I., & Mättö, J. (2004). Stationary-phase acid and heat treatments for improvement of the viability of probiotic lactobacilli and bifidobacteria. *Journal of Applied Microbiology*, *96*, 1205–1214.

Sagheddu, V., Elli, M., Biolchi, C., Lucido, J., Morelli, L., Technologies, A. A., et al. (2018). Impact of mode of assumption and food matrix on probiotic viability. *Journal of Food Microbiology*, *2*, 2–7.

Samona, A., & Robinson, R. K. (1994). Effect of yogurt cultures on the survival of bifidobacteria in fermented milks. *International Journal of Dairy Technology*, *47*, 58–60.

Santos, C. S. D., Kanup, R. F., Albuquerque, M. A. C., Bedani, R., Souza, C. H. B. D., Gioielli, L. A., et al. (2020). Effect of enzymatic interesterification on the textural and nutritional properties of a probiotic table spread containing milk fat. *LWT-Food Science and Technology*, *124*, 109129.

Saxelin, M., Lassig, A., Karjalainen, H., Tynkkynen, S., Surakka, A., Vapaatalo, H., et al. (2010). Persistence of probiotic strains in the gastrointestinal tract when administered as capsules, yoghurt, or cheese. *International Journal of Food Microbiology*, *144*, 293–300.

Semyonov, D., Ramon, O., Kaplun, Z., Levin-Brener, L., Gurevich, N., & Shimoni, E. (2010). Microencapsulation of *Lactobacillus paracasei* by spray freeze drying. *Food Research International*, *43*, 193–202.

Senaka Ranadheera, C., Evans, C. A., Adams, M. C., & Baines, S. K. (2012). Probiotic viability and physico-chemical and sensory properties of plain and stirred fruit yogurts made from goat's milk. *Food Chemistry*, *135*, 1411–1418.

Senz, M., van Lengerich, B., Bader, J., & Stahl, U. (2015). Control of cell morphology of probiotic *Lactobacillus acidophilus* for enhanced cell stability during industrial processing. *International Journal of Food Microbiology*, *192*, 34–42.

Settachaimongkon, S., van Valenberg, H. J. F., Winata, V., Wang, X., Nout, M. J. R., van Hooijdonk, T. C. M., et al. (2015). Effect of sublethal precultur-ing on the survival of probiotics and metabolite formation in set-yoghurt. *Food Microbiology*, *49*, 104–115.

Shafiee, G., Mortazavian, A. M., Mohammadifar, M. A., Koushki, M. R., Mohammadi, A., & Mohammadi, R. (2010). Combined effects of dry matter content, incubation temperature and final pH of fermentation on biochemical and microbiological characteristics of probiotic fermented milk. *African Journal of Microbiology Research*, *4*, 1265–1274.

Shi, X., Li, J., Wang, S., Zhang, L., Qiu, L., Han, T., et al. (2015). Flavor characteristic analysis of soymilk prepared by different soybean cultivars and establishment of evaluation method of soybean cultivars suitable for soymilk processing. *Food Chemistry*, *185*, 422–429.

Shimamura, S., Abe, F., Ishibashi, N., Miyakawa, H., Yaeshima, T., Araya, T., et al. (1992). Relationship between oxygen sensitivity and oxygen metabolism of *Bifidobacterium* species. *Journal of Dairy Science*, *75*, 3296–3306.

Shin, Y. J., Kang, C. H., Kim, W., & So, J. S. (2019). Heat adaptation improved cell viability of probiotic *Enterococcus faecium* HL7 upon various environmental stresses. *Probiotics and Antimicrobial Proteins*, *11*, 618–626.

Sloan, A. E. (2018). Top 10 functional food trends. *Food Technology*, *72*, 4.

Srisukchayakul, P., Charalampopoulos, D., & Karatzas, K. A. (2018). Study on the effect of citric acid adaptation toward the subsequent survival of *Lactobacillus plantarum* NCIMB 8826 in low pH fruit juices during refrigerated storage. *Food Research International*, *111*, 198–204.

Stack, H. M., Kearney, N., Stanton, C., Fitzgerald, G. F., & Ross, R. P. (2010). Association of beta-glucan endogenous production with increased stress tolerance of intestinal lactobacilli. *Applied and Environmental Microbiology*, *76*, 500–507.

Su, R., Zhu, X. L., Fan, D. D, Mi, Y., Yang, C. Y., & Jia, X. (2011). Encapsulation of probiotic *Bifidobacterium longum* BIOMA 5920 with alginate-human-like collagen and evaluation of survival in simulated gastrointestinal conditions. *International Journal of Biological Macromolecules*, *49*, 979–984.

Suomalainen, T., Lagström, H., Mättö, J., Saarela, M., Arvilommi, H., Laitinen, I., et al. (2006). Influence of whey-based fruit juice containing *Lactobacillus rhamnosus* on intestinal well-being and humoral immune response in healthy adults. *LWT-Food Science and Technology*, *39*, 788–795.

Talwalkar, A., & Kailasapathy, K. (2003). Metabolic and biochemical responses of probiotic bacteria to oxygen. *Journal of Dairy Science*, *86*, 2537–2546.

Talwalkar, A., & Kailasapathy, K. (2004). A review of oxygen toxicity in probiotic yogurts: Influence on the survival of probiotic bacteria and protective techniques. *Comprehensive Reviews in Food Science and Food Safety*, *3*, 117–124.

Tamime, A. Y., Saarela, M., Søndergaard, A. K., Mistry, V. V., & Shah, N. P. (2006). Production and maintaining viability of probiotic micro-organisms in dairy products. In A. Y. Tamime, & L. V. Thomas (Eds.), *Probiotic dairy products* (pp. 67–164). Hoboken, NJ: John Wiley & Sons.

Tompkins, T. A., Mainville, I., & Arcand, Y. (2011). The impact of meals on a probiotic during transit through a model of the human upper gastrointestinal tract. *Beneficial Microbes*, *2*, 295–303.

Tripathi, M. K., & Giri, S. K. (2014). Probiotic functional foods: Survival of probiotics during processing and storage. *Journal of Functional Foods*, *9*, 225–241.

Tymczyszyn, E., Gerbino, E., Illanes, A., & Gómez-Zavaglia, A. (2011). Galacto-oligosaccharides as protective molecules in the preservation of *Lactobacillus delbrueckii* subsp. *bulgaricus*. *Cryobiology*, *62*, 123–129.

Valerio, F., De Bellis, P., Lonigro, S. L., Morelli, L., Visconti, A., & Lavermicocca, P. (2006). In vitro and in vivo survival and transit tolerance of potentially probiotic strains carried by artichokes in the gastrointestinal tract. *Applied and Environmental Microbiology*, *72*, 3042–3045.

Valero-Cases, E., & Frutos, M. J. (2017). Effect of inulin on the viability of *L. plantarum* during storage and *in vitro* digestion and on composition parameters of vegetable fermented juices. *Plant Foods for Human Nutrition*, *72*, 161–167.

Vinderola, C. G., Mocchiutti, P., & Reinheimer, J. A. (2002). Interactions among lactic acid starter and probiotic bacteria used for fermented dairy products. *Journal of Dairy Science*, *85*, 721–729.

Waako, A., Polak-Berecka, M., & Gustaw, W. (2013). Increased viability of probiotic *Lactobacillus rhamnosus* after osmotic stress. *Acta Alimentaria*, *42*, 520–528.

Wang, W., Delettre, J., Corrieu, G., & Béal, C. (2011). Starvation induces physiological changes that act on the cryotolerance of *Lactobacillus acidophilus* RD758. *Biotechnology Progress*, *27*, 342–350.

Wang, W., Yu, R. C., & Chou, C. C. (2006). Antioxidative activities of soymilk fermented with lactic acid bacteria and bifidobacteria. *Food Microbiology*, *23*, 128–135.

Yeo, S. K., & Liong, M. T. (2011). Effect of ultrasound on the growth of probiotics and bioconversion of isoflavones in prebiotic-supplemented soymilk. *Journal of Agricultural and Food Chemistry*, *59*, 885–897.

Ying, D. Y., Schwander, S., Weerakkody, R., Sanguansri, L., Gantenbein-Demarchi, C., & Augustin, M. A. (2013). Microencapsulated *Lactobacillus rhamnosus* GG in whey protein and resistant starch matrices: Probiotic survival in fruit juice. *Journal of Functional Foods*, *5*, 98–105.

Zhang, C., Lu, J., Yang, D., Chen, X., Huang, Y., & Gu, R. (2018). Stress influenced the aerotolerance of *Lactobacillus rhamnosus* hsryfm 1301. *Biotechnology Letters*, *40*, 729–735.

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Chapter 29

Effective Probiotic Delivery: Current Trends and Future Perspectives

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1 Introduction

The consumption of probiotics is increasing due to numerous benefits conferred on the health of the consumers. A microorganism is considered as probiotics when it acts as a normal resident in the gastrointestinal tract (GIT) and survives the passage of the stomach, maintaining the viability and activity in the intestine (Cheng, Song, Xie, & Song, 2019). In addition, these microorganisms exhibit technological properties having good power of multiplication and promoting sensorial properties suitable in the product (Hammam & Ahmed, 2019; Zeng, Fan, He, Duan, & Xia, 2019). Probiotics should be stable and viable during storage, so that they can be manipulated and incorporated in the food products without loss of viability (Roberts et al., 2018).

The interest for the addition of probiotic microorganisms in the food product is growing day by day because of their nutritional and therapeutic values (Judkins, Archer, Kramer, & Solch, 2020). Foods with probiotics have great demand in the industrial market, and there is a tendency of consumption of these functional foods will increase over 50% in the coming years. However, there are some problems regarding the maintenance of the viability of probiotic microorganisms in some foods. Probiotic organisms show low viability due to various factors such as pH value, hydrogen peroxide and oxygen concentration, storage temperature, interaction with other microbes in the products, and lactic and acetic acid concentration, which affects the viability of these microbes in these foods (Dinkçi, Akdeniz, & Akalin, 2019; de Oliveira Ribeiro et al., 2020). Moreover, foods having low water activity and low hydrogen potential do not provide favorable conditions for their survival. Therefore, providing probiotics with a physical barrier to resist adverse environmental conditions is an approach that has received considerable attention in recent years.

The protection and viability of probiotic cultures during processing, storage, and passage of the product through the GIT are mandatory, which can be achieved through encapsulation technology (Liu et al., 2019). Encapsulation technique has the potential to serve as a useful tool to improve the survival of probiotics in the GIT as well as enhance the viability in food products. However, there is still some scope for the improvement in terms of probiotic survival at low pH, as well as focusing the research more toward the controlled release from the capsules. The nature of probiotic release may be tailored, which depends on the type of probiotic effect is intended to be exploited. The delivery of probiotics to the small intestine would favor immune response, whereas the colon is the traditional site of antipathogenic action (Valdés-Varela, Gueimonde, & Ruas-Madiedo, 2018; Hols, Ledesma-García, Gabant, & Mignolet, 2019).

Probiotics are strain-specific, and some strains do not have a resistance to environmental conditions like oxygen, light, and temperature. Thus, probiotic strains demand better protection for the development of new probiotic foods. In this case, microencapsulation has gained more attention to encapsulate probiotics during food processing and storage (Martín, Lara-Villoslada, Ruiz, & Morales, 2015). Microencapsulation has been reported to protect the probiotic organisms from adverse conditions of the digestive tract. The encapsulation process protects the functionality of the probiotics, which attributes targeted delivery in gut region (Speranza et al., 2017). The encapsulation material must be nontoxic and should have nutritional value, which could improve their applications in the food industry (Abd El-Salam & El-Shibiny, 2012). Polymers such as alginate, chitosan, starch, gelatin, pectin, xanthan gum, carrageenan, and carboxymethyl cellulose have been used as an efficient encapsulation material for probiotics. However, the successful probiotic encapsulation demands some improvements in the encapsulation technique as well as encapsulation materials. Microencapsulation of probiotics

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00029-0
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can be achieved using different techniques (emulsion, extrusion, coacervation, spray drying, and polymerization) and efficient materials to improve their performance in various fields of food and pharmaceutical industries (Shori, 2017).

Nanoencapsulation is an advanced method used to encapsulate sensitive probiotic strains. It has many advantages like narrow size, controlled release, and delivery in target sites due to its high immobilization efficiency, increased surface area to volume ratio, robust protection, and minimized sensorial effect when incorporated into food matrices (Feng et al., 2018; Šipailienė & Petraitytė, 2018). Thus, nanoencapsulation gained more interest in researchers to develop a new formulation for probiotics to enhance the production of functional probiotic foods.

There are many studies that have been published covering the different aspects of encapsulation. This chapter is a compilation of research updates on the potential role of encapsulated probiotics and their applications. Research prospects discussed in this chapter will encourage future researchers to focus on new technological attempts toward the development of advanced encapsulation technology.

2 Probiotics

2.1 Definition

Probiotics are live microorganisms that confer health benefits to humans and animals when consumed in an adequate amount. Probiotic foods also increase the interaction of resident microflora and their activity in the host system (Sanders, Merenstein, Merrifield, & Hutkins, 2018). The consumption of probiotic foods causes maintenance of beneficial microflora, which has an impact on the immune system, digestion, metabolism, and gut-brain communications (Kerry et al., 2018). Probiotics give protection against pathogenic bacteria and play an important role to keep us healthy (Siezen & Kleerebezem, 2011).

2.2 Health benefits

Probiotics exert several health benefits to human and animals such as the prevention of diseases and maintenance of healthy gut microbes. The ideal properties of probiotics include (1) digestion resistance, (2) adherence to intestinal wall, (3) stability in GIT conditions, (4) potentiality to produce antimicrobial compounds, (5) ability to restore/maintain normal microflora, and (6) nonpathogenic to the body (Sanap, Garje, & Godge, 2019) Adhesion properties of probiotic increase the colonization efficiency in the gut region, which could ultimately prevent pathogen colonization (Dlamini, Langa, Aiyegoro, & Okoh, 2019). Probiotics have an indirect inhibition mechanism for harmful bacteria by altering the pH and competing for oxygen and nutrients. Probiotics also produce bioactive and antimicrobial compounds that can prevent food spoilage and enhance the sensory attributes (Pop et al., 2020; Mukherjee et al., 2020; Ranadheera et al., 2019). Probiotics also produce some beneficial enzymes like β-galactosidase and lactase enzymes, which mitigate lactose intolerance (den Besten et al., 2013).

Healthy levels of total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides can be maintained after consumption of probiotic food. These cholesterol-lowering properties of probiotic strains could reduce the risk of cardiovascular disease and other dyslipidemia (Sivamaruthi, Kesika, Suganthy, & Chaiyasut, 2019). The efficacy of probiotics is also linked with the metabolic activity that can enhance the production of short-chain fatty acids (SCFAs) and vitamins Hati, Das, & Mandal, 2019. Human lacks number of carbohydrate digesting enzymes in their small and large intestine. Gut microbiota converts indigestible carbohydrate to hexose sugars, which can be utilized by probiotics to produce SCFAs. These SCFAs lower the pH of the gut to induce antimicrobial activities against pathogenic bacteria (Chugh & Kamal-Eldin, 2020; Schepper et al., 2017). Many probiotic strains such as *Propionibacterium shermanii*, *Bacillus subtilis*, and *Escherichia coli* produced vitamins like B_{12}, B-complex, riboflavin, propionic acid, folate, and thiamine (Piwowarek, Lipińska, Hać-Szymańczuk, Kieliszek, & Ścibisz, 2018; Masuda et al., 2012). Moreover, intestinal microbiota has a significant role in the production of vitamin K in humans (Patel, Shah, & Prajapati, 2013).

Probiotic foods encapsulated with prebiotic substances could improve digestive system and nutritional value. Several prebiotic substances like wheat bran, oats gum, and other cereals have been reported for their health-promoting benefits such as antioxidant, anticancer, and antiinflammatory activities (Górska, Przystupski, Niemczura, & Kulbacka, 2019; Molska & Reguła, 2019; Raman, Ambalam, & Doble, 2019; Ragavan & Das, 2018). Bioavailability of antioxidants could increase the free radical scavenging activity and help to control various diseases associated with oxidative stress (Romero-Luna, Hernández-Sánchez, Ribas-Aparicio, Cauich-Sánchez, & Dávila-Ortiz, 2019). Probiotic strains can be used to prevent autoimmune and inflammatory diseases (Salami, Kouchaki, Asemi, & Tamtaji, 2019). Secretion of immunomodulatory compounds and metabolites may positively modulate the immune system (Nazir, Hussain, Abdul Hamid, & Song, 2018).

The therapeutic properties of probiotics can be used for the prevention/treatment of colon cancer. Colon cancer is mainly associated with human's diet and lifestyle. The dietary control plays a significant role in the prevention of cancer. So probiotics can serve as a dietary supplement, which may have beneficial effects on colon cancer. Also, probiotic delivery could modulate the gut microbiota, which stimulates anticancer activity in the gut region. The metabolites produced by probiotics have suppressive activity on colon cancer cells, which includes acetate, propionates, butyrates, and SCFAs. Butyrate found to have antitumor activity, which induces apoptosis and inhibits proliferation and modifications in the expression of gene and cytokines (Schepper et al., 2017).

Another beneficial effect of probiotic is the prevention or treatment of gastrointestinal diseases such as intestinal diarrhea and lactose indigestion. Probiotic formulations are used to treat diarrhea, ulcer, and Crohn's disease. Probiotics like *Lactobacillus plantarum* are used to treat flatulence and abdominal pain. *Lactobacillus rhamnosus* GG, *Lactobacillus reuteri*, and *Saccharomyces boulardii* have been used for the control of GI disorders such as diarrhea, paining, and bloating (Pandey, Naik, & Vakil, 2015; Iannitti & Palmieri, 2010). Probiotics strains *Lactobacillus brevis*, *Lactobacillus casei*, *Lactobacillus vaginalis*, *Lactobacillus salivarius*, *L. reuteri*, *Lactobacillus delbrueckii*, and, *L. rhamnosus* have been reported to cure urinary tract infections. Probiotics can modulate the gut hormones such as gastric inhibitory polypeptide along with glucagon and peptides, which can be used to treat Type-2 diabetes as well as obesity (Westfall, Lomis, & Prakash, 2018; Chua, Kwok, Aggarwal, Sun, & Chang, 2017; Bermudez-Brito, Plaza-Díaz, Munoz-Quezada, Gomez-Llorente, & Gil, 2012).

Probiotic strain *L. plantarum* L67 produced interleukin-12 and interferon-γ in the host system, which can be used to treat disease/disorders, which is associated with antiallergy activity (Sartor, 2004). Gut microflora has a significant effect on the central nervous system, which can be used to treat autism and attention-deficit/hyperactivity disorder (Schepper et al., 2017). *L. rhamnosus* GG, a probiotic strain, could modulate the immunity by affecting the proliferation of murine T and B lymphocytes. The production of nitric acid and cytokines by probiotics also modulates the immune responses (Wang, Yao, Lv, Ling, & Li, 2017; Alvarez-Olmos & Oberhelman, 2001). The beneficial effects of probiotics have been extended to areas like mood improvement, vaginal health, and metabolism homeostasis (Cheng, Song, Xie, & Song, 2019). Therefore, probiotic foods gained more attention to the consumers and pose a great demand in the market.

3 Selection of probiotic strains for technological performance

Probiotic microorganisms are influencing the food products due to several technological factors based on their physiological traits such as oxygen tolerance, acid tolerance, bile salt tolerance, and heat tolerance (Bhadoria & Mahapatra, 2011). The processing and storage capacities of probiotics are often strain-specific (Ren et al., 2019). High levels of oxygen in food products are highly harmful to probiotic strains. Oxygen toxicity significantly affects the survival of probiotic strains in dairy foods (Talwalkar, Kailasapathy, Peiris, & Arumugaswamy, 2001).

3.1 Oxygen tolerance

The human gut microbiota contains different species of microbes that include both aerobic and anaerobic organisms. Anaerobic bacteria are dominant in the gut region and play a vital role in human health, which includes essential metabolite production and their regulation. *Bifidobacteria* is the most dominant anaerobic bacteria in human GIT and widely used in the production of probiotic products for infants, children, and adults. Probiotic bacteria lack oxygen scavenging ability, which results in reduced viability in food products. In yoghurt, protection of *Bifidobacteria* from oxygen was improved by adding *Streptococcus thermophilus*, which consumes high oxygen from the foods (El Enshasy et al., 2016). In contrast, probiotic strains belonging to the aerobic condition can survive in the presence of oxygen. Many Lactic acid bacteria (LAB) strains have oxygen tolerance capacity. The consumption and utilization of oxygen activate the respiratory metabolism in *Lactobacillus gasseri*, which yield high biomass production. These strains can be useful in the food industry as a starter culture for technological and health-promoting applications (Maresca, Zotta, & Mauriello, 2018).

3.2 Acid tolerance

Survival under lower acidic conditions during GIT transit is the first challenge encountered by the probiotic strains as the human stomach contains gastric acid, which maintains the acidity level (pH) around 1.5-3 (Hassan, Hung, & Sarbini, 2019). Probiotic strains must have acid tolerance ability to withstand the extremely acidic conditions at the gut region. Many probiotic strains especially LAB have acid tolerance capacity and play a crucial role in the fermentation industries (Fontana et al., 2019). Fermented food products are manufactured with the specific acidic condition and fermented by probiotic

bacteria/yeast (Panghal et al., 2019). LAB such as *L. brevis*, *Lactobacillus fermentum*, and *Leuconostoc mesenteroides* are used for the fermentation of durian fruit. Probiotic strains, namely, *L. plantarum*, *Lactobacillus pentosus*, *L. mesenteroides*, *Pediococcus cerevisiae*, and *L. brevis* are used for the preparation of the olive probiotic drink. The vegetable-based probiotic products like kanji (carrot, beetroot), sauerkraut (cabbage), kimchi (cabbage and radish), and kombucha (tea leaves) are also fermented by probiotic strains of LAB (Er, Tosun, Berk, & Kivanc, 2019).

3.3 Bile acid tolerance

Bile tolerance is another desirable property for potential probiotics. Bile acids are synthesized from cholesterol in liver hepatocytes present in humans and animals. It acts as a biological detergent to eliminate the cholesterol. It can emulsify and solubilize dietary lipids. Usually, human liver secretes 0.2%-2% bile followed by food ingestion, and probiotic strains must ensure the viability during bile exposure in the intestine. Bile tolerance also improves the colonization ability in the GIT Hu, Yuan, Yue, & Guo, 2018. In addition, bile tolerance reduces the colonization of pathogens, which includes disruption of macromolecule stability, membrane damage, and disappearance of bacterial transmembrane electrical potential (Bustos, de Valdez, Fadda, & Taranto, 2018). The enzyme responsible for bile hydrolysis is choloylglycine hydrolase (intracellular enzyme), which breaks the amide bond between glycine and the steroid nucleus of bile acids. Bile salt hydrolysis (BSH) achieved through enzymatic hydrolysis by several probiotics belonging to *Lactobacillus* spp. and *Bifidobacterium* spp. BSH activity by microbes also increased the amino acids content and used as a carbon/nitrogen source for their growth. Increased biomass and BSH activity improve the detoxification of bile and cholesterol-lowering effects. The mechanism behind the bile tolerance is strain-specific, which includes alternations in the cell wall, active efflux pump, and BSH activity (Horáčková, Plocková, & Demnerová, 2018). Microbes need more energy, which is obtained from carbohydrate metabolism to maintain the cellular process under stress conditions. During bile exposure, microbes alter the pathways of carbohydrate metabolism, which have been confirmed at proteome level in few probiotic strains such as *Bifidobacterium longum*, *Enterococcus faecalis*, and *L. reuteri*. This alteration could modify the cell envelope, prevent protein misfolding, and mediate bile efflux by adjusting carbohydrate metabolic pathways. Hence, bile stress not only affects probiotic traits but also significantly alters other attributes such as improvement of the generation of commodities resulting from metabolic processes, which may be valuable in food technology (Ali, Singh, Tomar, Mohanty, & Behare, 2020).

3.4 Temperature tolerance

Temperature is the primary factor affecting the technological performance of probiotic strains during processing, manufacturing, and storage. The human gut temperature is 37°C, and the resident gut microbiota could survive at this condition in humans and other animals. In the case of probiotic product development, strains should tolerate much higher temperatures around 60°C-67°C. Heat tolerance ability ultimately improves the stability of probiotics throughout their shelf life (Dinkçi, Akdeniz, & Akalin, 2019; Shin, Kang, Kim, & So, 2019). Heat stress treatment for probiotics could be a promising strategy to improve their shelf life in fermented foods. Mild heat stress treatment retains the viability of probiotics in fermented milk products (Zhang, Li, Cheng, Buch, & Zhang, 2019). Probiotic bacteria/yeasts are healthy to feed additives at aquaculture and poultry farms. Probiotic dietary supplementation in poultry maintains the appropriate number of probiotic strains under heat stress conditions (Ebeid, Fathi, Al-Homidan, Ibrahim, & Al-Sagan, 2019; Sugiharto et al., 2019).

The use of probiotic bacteria in food products still encounters many challenges related to their viability, survival, and stability. It also affects the functionality of food during processing and storage, which includes consumption and sensory changes of probiotic foods (Min, Bunt, Mason, & Hussain, 2019). Although many strains have been reported as potential probiotics, only a few strains are being used for their technological performance at different conditions. The technical problems affect the efficacy of probiotics as well as food quality, which may reduce the acceptability of probiotic foods to the consumers. Alternative methods can be used for the processing and storage conditions like encapsulation technique, addition of various protectants, and the use of oxygen barrier packaging materials to retain the shelf life of probiotics in the food products. The interactions between probiotics and different food matrices will improve the emergence of new probiotic products in the market (Dinkçi, Akdeniz, & Akalin, 2019). Hence, we need to focus on encapsulation technology to improve probiotic functional foods.

4 Use of encapsulation technology for effective delivery of probiotics

The increased world's population highly demands functional foods that contain probiotics and natural active compounds like polyunsaturated fatty acids, vitamins, and minerals for the consumption of human and animals. The active compounds are not stable under certain conditions or have limited applications in the food industry (Chew et al., 2019). Moreover,

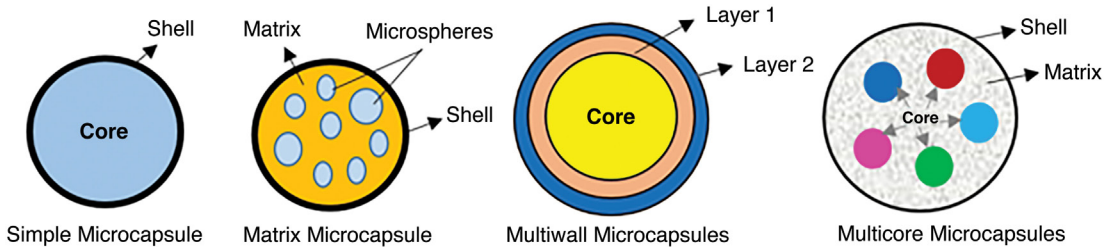


FIGURE 29.1 Types of encapsulation methods.

probiotics lack their viability and characteristic features in food products during processing, storage, and GIT transit followed by the ingestion, which is a major problem in the food industry (Rodrigues, Silva, Mulet, Cárcel, & Fernandes, 2018). According to WHO/FAO, probiotic foods should have viable cells around 10^6-10^7 CFU/g at the time of consumption. Therefore, it is necessary to use effective techniques to protect the functional stability of probiotics, which will enable better absorbance in our GIT as well as improve their applications in the food industry. Probiotic strains are encapsulated with various materials/protectants to withstand harsh environmental conditions. The probiotic bacteria, which play a significant role in the food industry, mostly belong to the genera of *Lactobacillus* and *Bifidobacterium* (Dinkçi, Akdeniz, & Akalin, 2019).

Encapsulation is an effective technique used to protect the probiotics at different conditions. Usually, probiotics are mixed with core material and then coated with biodegradable protective materials to produce microcapsules. Different types of encapsulation methods have been shown in Fig. 29.1. Addition of one or more protective layers on the probiotics could enhance the probiotic delivery effectively. In this method, core material holds probiotics and coating material improves the viability of probiotics during GIT transit and stability in food products (Pech-Canul, Ortega, García-Triana, & González-Silva, 2020). Incorporation of probiotics in food matrices such as chocolates, cereal products, beverages, and vegetables gives better protection to probiotics during processing, storage, as well as GIT transit (Terpou et al., 2019). Food components such as carbohydrate and protein-based encapsulation have a significant protective effect on probiotic during dehydration, GIT transit, and storage (Dianawati, Mishra, & Shah, 2016). The common encapsulation techniques are shown in Table 29.1.

TABLE 29.1 Encapsulation techniques widely used for probiotics.

Encapsulation techniques	Particle size (μm)	Advantages	Disadvantages	References
Polymerization-complex emulsion	1-1000	Easy and uniform coating	Agglomeration due to separation	Abdelkader, Hussain, and Abdullah (2018)
Air suspension coating	35-5000	Suitable for micron and submicron particles	Applicable for only solids	Begum et al. (2018)
Solvent evaporation	1-5000	Controlled release at specific targets	Difficult to control evaporation rate	da Silva et al. (2018), Alli (2013)
Multiorifice centrifugal process	1-5000	High production rate and applicable for both solid and liquids	Wastage of coating material and needs high temperature to dry particles	Khan et al. (2015), Kumar, Tejbe, Banu, Lakshmi, and Bhowmik (2013)
Pan coating	600-5000	Easy and low cost and enhanced released	Time-consuming process and huge material loss	Soukoulis et al. (2014), Semyonov, Ramon, Kovacs, Friedlander, and Shimoni (2012)
Coacervation-phase separation	2-5000	High encapsulation efficiency and versatile process	Instability in aqueous solutions and difficult to scale up	Ezhilarasi, Karthik, Chhanwal, and Anandharamakrishnan (2013)
Ionotropic gelation	5-15	Sustained release properties and uniform distribution	High cost and changes for clogging	Patil et al. (2012)
Spray drying	600	Cheap and flexible process	High capital cost and variations in particle size	Fang and Bhandari (2010)

In addition to these traditional methods, new techniques such as nanoemulsions, PEGylation, bioactive tagging, and electrospinning for producing nanofibers and nanotubes have also been developed Ozdal, Yolci-Omeroglu, & Tamer, 2020. In nanoemulsions, two immiscible liquids are dispersed in small spherical droplets to produce stable colloidal dispersion. The smaller droplet size could improve the bioavailability of probiotics in functional beverage products (de Oca-Ávalos, Candal, & Herrera, 2017). PEGylation is a novel method mostly used for the targeted delivery of probiotics and other compounds. The covalent bond between polyethylene glycol (PEG) and probiotics provides stability in the food products (Mishra, Nayak, & Dey, 2016). Electrospinning is a flexible method used to encapsulate probiotics by applying an electric field to draw continuous fibers. It enhances the encapsulation efficiency and produces fibers with high porosity and surface-to-volume ratio. There is no heat application in this method, which could preserve the viability of sensitive probiotics during the spinning process (Wen, Zong, Linhardt, Feng, & Wu, 2017).

4.1 Significance of cell survival during processing and storage

In recent years, the development of probiotic food products using new technology or formulation gained more attention to the consumers. The survival of probiotics in food products is affected by several stress conditions like osmotic, oxidative, heat, and cold stresses during processing and storage (Amund, 2016). The new formulation or technology for making probiotic foods may improve the survival of probiotics during the various manufacturing process such as mixing, drying, threshing, winnowing, shelling, and milling (Roobab et al., 2020). Therefore, probiotic strain should have the ability to withstand these technological stress conditions.

The survival of bacteria or yeast is a prerequisite for probiotics in functional foods. It ensures the functionality of probiotics in food products. Immobilization/encapsulation is a technique widely used to improve the survival rate of probiotics in food products. Immobilization/entrapment of probiotics in bacterial cellulose increased the survival of probiotics in white brined cheese during preservation (Moghanjougi, Bari, Khaledabad, Almasi, & Amiri, 2020). Probiotic bacteria could reduce aflatoxin contamination in some of the milk products (Serrano-Niño et al., 2013). Alginate is a biopolymer and considered as a suitable material for the entrapment of immobilized probiotic yeast, which improves the absorbance of aflatoxin (Farbo et al., 2016). The delivery of probiotics may differ based on vehicle nature used for the encapsulation process. Different materials have been used to encapsulate probiotic strains to improve their performance in functional food products.

Probiotic metabolites like exopolysaccharides and fatty acids can serve as a vehicle for the delivery of probiotics in various food products (Song et al., 2019). The food matrix or pharmaceutical particle should have the ability to deliver viable probiotic cells during manufacture as well as storage (Terpou et al., 2019). Probiotics are incorporated into edible thin-layer films to enhance the survival rate during the development of functional foods. For example, *L. plantarum* KMC45 encapsulated in alginate/pectin films showed improved survival during the drying process (Shahrampour, Khomeiri, Razavi, & Kashiri, 2020). Incorporation of probiotics in *Pistacia terebinthus* resin showed retained shelf life in cheese products after the process of manufacture (Schoina et al., 2020). Emulsion microgel particles are a novel encapsulation strategy used for the encapsulation and delivery of sensitive probiotics (*Pediococcus pentosaceus*) in functional foods and medicine (Yao et al., 2018).

The selection of cryoprotective agent also has a significant influence on probiotic survival in lyophilized products (de Moraes, dos Santos, de Barcelos, Lopes, & do Egito, 2018). The cryoprotective agents such as proteins, carbohydrates, and polymers are used for the encapsulation of probiotics during their preservation. The mixture of cryoprotectants and their synergistic effects could enhance probiotic survival during the freeze-drying process of milk products (Ren et al., 2019; Nelson et al., 2019; Xavier, Madhankumar, Natarajan, Ramana, & Semwal, 2019). The survival of probiotics during refrigeration storage could maintain the quality of food products like cheese, butter, yoghurt, and other dairy products (Ningtyas, Bhandari, Bansal, & Prakash, 2019).

4.2 Significance of cell survival during GIT transit

The passage of probiotics in GIT is not smooth, and physiological properties (namely, low pH, bile salts, digestive enzymes, and peristaltic motion) can affect their survival (Boricha, Shekh, Pithva, Ambalam, & Vyas, 2019). Many encapsulation techniques enhance the survival of probiotics during GIT transit (Champagne, da Cruz, & Daga, 2018). Traditional delivery of probiotics mostly associated with fermented milk products. Dairy-based food matrix maintained the sufficient viable cell count (10^6-10^7 CFU/mL) and found to be an effective carrier for probiotics (Ranadheera et al., 2019). Incorporation of diary substrate in probiotics could improve the acid tolerance ability of probiotics during their transit (Archacka et al., 2019). Probiotic strains survive in gastric acid during their transit and also enable the colonization and proliferation ability in host used for the prevention of gastrointestinal diseases (Shori, 2017). Sodium alginate and chitosan-based encapsulation

provides more protection to probiotics during their transit. Especially, sodium alginate with cow milk or goat milk improved their performances in simulated GIT conditions (Prasanna & Charalampopoulos, 2018). Double-layered encapsulation technique is an efficient method to improve the survival rate of probiotics under harsh GIT conditions (Feng et al., 2020). Many compounds have been used for the encapsulation of probiotics, such as polysaccharides, proteins, and plant and animal gums.

Microencapsulation of probiotics using a polysaccharide-protein matrix (whey protein-alginate) and alginate-chitosan matrix could enhance their survival during GIT transit (Iqbal, Zahoor, Huma, Jamil, & Ünlü, 2019; Krunić, Obradović, & Rakin, 2019). Probiotic bacteria *Lactobacillus bulgaricus* and *Lactobacillus paracasei* encapsulated with whey protein isolate and xanthan gum exhibited improved survival rate under simulated GIT conditions. The pH buffering capacity of whey protein isolate and xanthan gum creates a physical barrier between the cell wall and acid environment, which could improve the survival of probiotics during their GIT transit (Vargas, Olson, & Aryana, 2015; Xiao et al., 2020a). Probiotic strain encapsulated in emulsion with multilayers (lipid-protein-pectin) increases the survival rate of probiotics under harsh GIT conditions (Zhang, Wu, & Fei, 2016). Thus, encapsulation technique provides better protection to probiotic strains, which could maintain the stability and viability of probiotics during their GIT transit.

4.3 Improvement of sensory characters and limitations

In functional foods, aroma and flavors are important ingredients and it may get affected during food processing and storage. The clarification of fruit juices, controlled release of aroma, foaming, enhancing aroma and flavor, and the color of beverages were improved by encapsulation technology (Ozdal, Yolci-Omeroglu, & Tamer, 2020). Some cyclic oligosaccharides (such as cyclodextrins) act as a physical barrier to aroma and flavor compounds to protect their stability during heating and reactions with ingredients (Pereira, Marques, Hatano, & Castro, 2010). Probiotic strains improve the sensory properties of the food products during processing at different conditions. The sensory properties of cheese products fermented with *Bacillus* strains, namely, *B. subtilis*, *Bacillus coagulans*, and *Bacillus flexus*, and *Lactobacillus* strains, namely, *L. casei*, *L. plantarum*, and *L. brevis*, remained unaffected throughout the manufacturing process (Ningtyas et al., 2019; Soares et al., 2019; Mushtaq, Gani, & Masoodi, 2019; Reale, Di Renzo, & Coppola, 2019).

In recent years, the development of functional beverages from fruits and vegetables has been increased throughout the world. The probiotic-incorporated beverages attract wide range of consumers. Incorporation of probiotics in the fermented dairy-based beverage with the addition of mango juice improved the viability as well as sensory scores (Ryan et al., 2020). Encapsulation of probiotics may suppress the undesirable flavors and aromas of the end products. The enhancement of the sensory effects and reduction of the acidification in orange juice were observed in encapsulated *L. rhamnosus* GG and *Lactobacillus acidophilus* NCFM (Sohail, Turner, Prabawati, Coombes, & Bhandari, 2012). Many studies suggested that encapsulation can be used to reduce the negative sensory effects in some of the fruit juices such as apple, orange, grape, and pineapple (Gandomi, Abbaszadeh, Misaghi, Bokaie, & Noori, 2016; Krasaekoopt & Kitsawad, 2010; Phoem, Chanthachum, & Voravuthikunchai, 2015). Therefore, encapsulation of probiotics can be used to enhance the sensory properties of the food products.

5 Microencapsulation of probiotics

The production of probiotic foods at industrial scale encountered many technological, economic, and microbiological challenges. The stability of probiotic strains under different conditions as well as during GIT transit requires appropriate carrier matrices and effective technological design. Encapsulation of probiotics can be applied in a variety of foods with functional properties (Terpou et al., 2019). Microencapsulation is an efficient method to develop a novel and sustainable formulations using various biopolymers such as alginate, chitosan, gelatin, whey protein isolate, and cellulose derivatives (Šipailienė & Petraitytė, 2018).

5.1 Efficient matrices for microencapsulation of probiotics

The ideal encapsulation material should have the ability to seal the microcapsule that contains probiotics and provides robust protection from harsh conditions. Several polysaccharides such as maltodextrin (from vegetable starch), gum arabic (from Acacia tree), chitosan (from shrimp and crustaceans), alginate (from brown microalgae), and microbial polysaccharides (gellan and xanthan) have been used as active carriers for probiotic strains. *K*-Carrageenan is a natural polymer that ensures excellent viability of probiotics after encapsulation and widely used in the food industry (Ozdal, Yolci-Omeroglu, & Tamer, 2020). Proteins such as casein, whey, and pectin are also used for the encapsulation of probiotics. Whey protein

TABLE 29.2 Efficient matrices for encapsulation of probiotics.

Core material	Coating material	Probiotics	References
Alginate	Chitosan	*Lactobacillus rhamnosus* GG	Qi, Simsek, Ohm, Chen, & Rao, 2020
	Whey protein	*Lactobacillus acidophilus*	de Araújo Etchepare et al. (2020)
	Gellan	*Lactobacillus plantarum* CECT 220	González-Ferrero et al., 2020
	Gelatin	*L. acidophilus* FNcc-0051	Diana and Purwanti (2019)
	K-Carrageenan	*L. acidophilus*	Afzaal et al. (2019)
	Resistant starch	*L. plantarum* and *Bifidobacterium lactis*	Zaeim, Sarabi-Jamab, Ghorani, and Kadkhodaee (2019)
	Inulin	*Bifidobacterium* sp.	Pradeep Prasanna and Charalampopoulos (2019)
	Carboxymethyl cellulose	*Lactococcus lactis*	Ye, Ma, Qin, and Liu (2018)
	Poly-L-lysine	*Lactobacillus casei* and *Bifidobacterium adolescentis*	Zanjani et al. (2018)
Pectin	Alginate	*Bifidobacterium longum* BL-05	Yasmin, Saeed, Pasha, and Zia (2019)
	Starch	*Lactococcus raffinolactis* and *Lactobacillus paracasei*	Coqueiro, Raizel, Bonvini, Tirapegui, and Rogero (2019)
	Whey protein	*Lactobacillus* spp.	Eckert et al. (2018)
	Gelatin	*L. plantarum*	Pavli, Tassou, Nychas, and Chorianopoulos (2018)

is mostly used as a wall material and can be used to encapsulate products that lack emulsification properties (Donsì et al., 2019. Table 29.2 shows the efficient materials widely used for the encapsulation of probiotics.

Agarose was used as an efficient matrix for encapsulation of probiotics, but it has a gelling point at 40°C. Agarose was combined with other hydrocolloids (alginate, gelatin, and whey protein) to produce probiotic-hydrogel particles to improve their performance. This method could be useful to encapsulate and deliver sensitive probiotic strains (*Bifidobacterium pseudocatenulatum* CECT 7765 and G7) in functional foods (Alehosseini & Jafari, 2019; Gu et al., 2019). Encapsulation using one or more polysaccharides like sodium alginate and carrageenan could improve the viability of probiotics (*L. acidophilus*) under simulated GIT conditions in ice creams (Afzaal et al., 2019). Probiotics encapsulated with disaccharides could improve the survival rate at different storage conditions. Alginate-starch microcapsule further coated with chitosan showed better protection and effective delivery under harsh conditions (Agudelo et al., 2017). The combination of polysaccharide and protein also contributes to electrostatic interactions, which could improve the thermal stability of microcapsules (de Araújo Etchepare et al., 2020). Alginate and buttermilk protein could serve as a carrier for probiotic bacteria *L. rhamnosus* GG (Hugues-Ayala, Sarabia-Sainz, González-Rios, Vázquez-Moreno, & Montfort, 2020).

The matrix of sodium caseinate and gum arabic/gum ghatti improved the viability of probiotic cells during GIT transit and storage in *L*actobacillus *zeae* LB1 (Liu et al., 2019). Probiotic entrapment using gelatin and carboxymethyl cellulose could increase the survival rate of probiotics during their GIT transit (Singh et al., 2019). Entrapment of *L. casei* ATCC393 using *P. terebinthus* (viscus matrix) resin showed retained viability and antimicrobial properties in cheese products (Schoina et al., 2020) Core-shell microgels obtained from cellulose (core) and calcium alginate (shell) are found to have stable viability during simulated GIT exposure (Liu et al., 2019). *Bifidobacterium bifidum* encapsulated in zein-coated alginate microgels shows enhanced survival rate under simulated GIT conditions (Riaz et al., 2019).

5.2 Effective encapsulation methods

Encapsulation of probiotics is achieved by various techniques. Extrusion (layer-by-layer approach) and coacervation methods are widely used for industrial applications. In extrusion, multilayer encapsulation (layer-by-layer approach) promotes protection to probiotics during processing and storage (Pitigraisorn, Srichaisupakit, Wongpadungkiat, & Wongsasulak, 2017). The formation of multilayer biopolymers achieved by electrostatic self-assembly may improve the encapsulation efficiency and stability of encapsulated probiotics during storage. Layer-by-layer self-assembly with $CaCl_2$ cross-linking provides higher viability in the gastric region. Multilayer capsule has different chemical properties that can provide strong

thermotolerance to probiotic foods during different stages of processing and storage. Probiotic bacteria *L. rhamnosus* GG encapsulated with multilayers of biopolymers and proteins enhance the performance of probiotics in acidic conditions during their GIT transit (Yucel Falco et al., 2019; Luo et al., 2020). Probiotic strain *L. rhamnosus* GG encapsulated in polyalginate with multiple polyelectrolyte layers shows increased viability under simulated GIT conditions (Eshrati et al., 2018).

L. casei and *Bifidobacterium adolescentis* microencapsulated in various starches (calcium alginate, wheat, rice, and high-amylose corn) along with chitosan and poly-L-lysine coating promote their survival rate during the production of ice creams (Zanjani, Ehsani, Ghiassi Tarzi, & Sharifan, 2018). Probiotic bacteria *L. brevis* WK12 and *Lactococcus lactis* WK11 encapsulated with protein and polysaccharides showed retained viability during long-term storage. The bilayered and multilayered vehicle presents a significant potential in encapsulation of probiotics and provides more resistant ability under harsh conditions (Table 29.3).

Complex coacervation is a technique used to encapsulate probiotics for effective delivery due to its advantageous like high cell-entrapment efficiency, improved functional performance, and good suitability for large-scale production (De Prisco & Mauriello, 2016; Zhao, Ling, Zhang, Ke, & Hong, 2018). This method can be used to promote the efficient release of encapsulated probiotics from encapsulated biopolymer microgels (Bosnea, Moschakis, Nigam, & Biliaderis, 2017). Alginate-gelatin microgels showed retained viability of *L. salivarius* Li01 during long-term storage after high-temperature treatments and during their GIT transit (Yao et al., 2018). Probiotic microgels along with cellulose nanoparticles significantly enhance the resistance of probiotics under simulated gastric conditions by filling the pores and thereby reducing the entry of gastric acid (Gopinath, Saravanan, Al-Maleki, Ramesh, & Vadivelu, 2018).

TABLE 29.3 Recent reports on microencapsulation for probiotics.

Probiotics	Matrices	Remarks	Reference
Lactobacillus bulgaricus and *Lactobacillus paracasei*	Whey protein and xanthan gum	Improve thermal stability	Xiao et al. (2020b)
Lactobacillus acidophilus	Alginate, starch, chitosan	Retained viability during GIT transit	Liu et al. (2019)
Pediococcus pentosaceus	Alginate, pectin, chitosan	Enhance the survival under GIT conditions	Yodsenee et al. (2020)
Lactobacillus pentosus	Chitosan, sodium phytate	Improves viability during storage	Wang et al. (2019)
L. acidophilus	High maize starch, maltodextrin, and gum arabic	Refrigeration storage for long duration	Reyes, Chotiko, Chouljenko, and Sathivel (2018)
Lactobacillus casei and *Bifidobacterium adolescentis*	Starch, chitosan and poly-L-lysine	Used to coat ice creams	Zanjani et al. (2018)
L. casei, *Lactobacillus brevis*, and *Lactobacillus plantarum*	Alginate, resistant starch	Improve the survival of probiotics during GIT simulation	Ashwar, Gani, Gani, Shah, and Masoodi (2018)
Lipomyces starkeyi VIT-MN03	Alginate, oats bran gum, and whey protein	High survival at adverse GIT conditions	Ragavan and Das (2018)
Lactobacillus rhamnosus	Alginate and gelatin	Increased viability during GIT transit and storage	Lopes, Bueno, Aguiar Júnior, and Finkler (2017)
L. rhamnosus	Whey protein and maltodextrin	Improves shelf life of probiotics in food products	Agudelo et al., 2017
L. rhamnosus	Alginate, cellulose nanocrystal, and lecithin	Enhances viability of probiotics during freeze-drying	Huq et al. (2017)
L. casei	Alginate, soya protein isolate	Suitable for functional probiotic foods	Hadzieva et al. (2017)
L. brevis WK12 and *Lactococcus lactis* WK11	Alginate, skim milk, soy powder, yeast extract, and trehalose	Cryoprotective agents served as a potential matrix for long-term storage	Gwak et al. (2015)
B. adolescentis	Alginate, pea, soy, faba, and lentil protein	Better survival in commercial fruit juices	Wang, Korber, Low, and Nickerson (2015)
Streptococcus salivarius sp. *thermophilus*, *Lactobacillus*, and *Bifidobacterium*	Alginate, chitosan	Freeze-dried microcapsules used as a starter culture in fermented whey beverages	Obradović et al. (2015)

Edible polymers are well established and eco-friendly materials used for the encapsulation of probiotics over synthetic polymers. This could reduce environmental contamination and improve the recyclability of materials (Shit & Shah, 2014). The widely used edible coating materials for the encapsulation process include proteins (soy, gelatin, and collagen) and polysaccharides (starch, chitosan, alginate, and cellulose derivatives). Edible cellulose-based films are used to develop novel probiotic products (Riaz et al., 2019). Probiotic strains such as *L. paracasei* and *Lindnera saturnus* showed improvements in the composition of functional beverages (Vong & Liu, 2019).

Probiotics are encapsulated with sufficient amount of beneficial nutrients (prebiotic) to enhance the survival of probiotics during storage and GIT transit within the human gut. The combination of probiotics and prebiotics is called synbiotics (Liao, Liu, Wang, Su, & Liu, 2019). Synbiotics are a promising area of research to design new functional foods with the combination of probiotics and prebiotics (cereals, fatty acids, and vitamins). It can modulate the microbiota and confer health benefits in humans. Cereal-based probiotic food products such as wheat, maize, oat, and barley are having abundant sources of dietary fiber and used for microencapsulation of probiotics due to their beneficial physiological effects on the gut (Min et al., 2019). Probiotic yeast such as *Kluyveromyces lactis* VIT-MN02, *Lipomyces starkeyi* VIT-MN03, and *Saccharomycopsis fibuligera* VIT-MN04 incorporated in alginate-oats bran gum along with whey protein coating promotes the survival of probiotics under simulated GIT conditions (Ragavan & Das, 2018). Psyllium and fenugreek have potential properties as efficient prebiotics, which could enhance the viability as well as growth of probiotics in the gut region (Haghshenas et al., 2015). Probiotic bacteria *L. rhamnosus* GG encapsulated in pectin microgels show improved viability in simulated GIT conditions (Kwiecień & Kwiecień, 2018). Similarly, *Weissella cibaria* JW15 encapsulated in biodegradable and biocompatible materials like alginate and chicory root extract along with PEG showed better protection after simulated GIT treatment. This formulation is considered as a promising delivery system for probiotic cells Kim, Nam, Im, Choe, & Choi, 2020. Encapsulation of *L. acidophilus* in alginate-whey protein isolate would improve the survival rate of probiotic bacteria under simulated GIT conditions (Dehkordi, Alemzadeh, Vaziri, & Vossoughi, 2020). These findings suggest that the choice of matrices plays an important role for the encapsulation of probiotics in functional food products.

6 Nanoencapsulation of probiotics

Nanoencapsulation is a desirable methodology used to develop a variety of probiotic food products. It is used to design a new formulation to deliver the probiotics to specific targeted regions like the small and large intestine. Moreover, nanomaterial increases the efficacy, specificity, and targeting ability of probiotics due to its small size, which could pass the probiotics rapidly through the intestinal wall to the body cells. Nanoencapsulation has many advantages such as small size, improved shelf life, and stability of probiotics under stress conditions. In addition, it needs only less active ingredients during the process of encapsulation (Kumar et al., 2018; Fung, Yuen, & Liong, 2011). The classification of nanoencapsulation techniques is divided into four groups based on the encapsulation method and their vehicle type (Fig. 29.2).

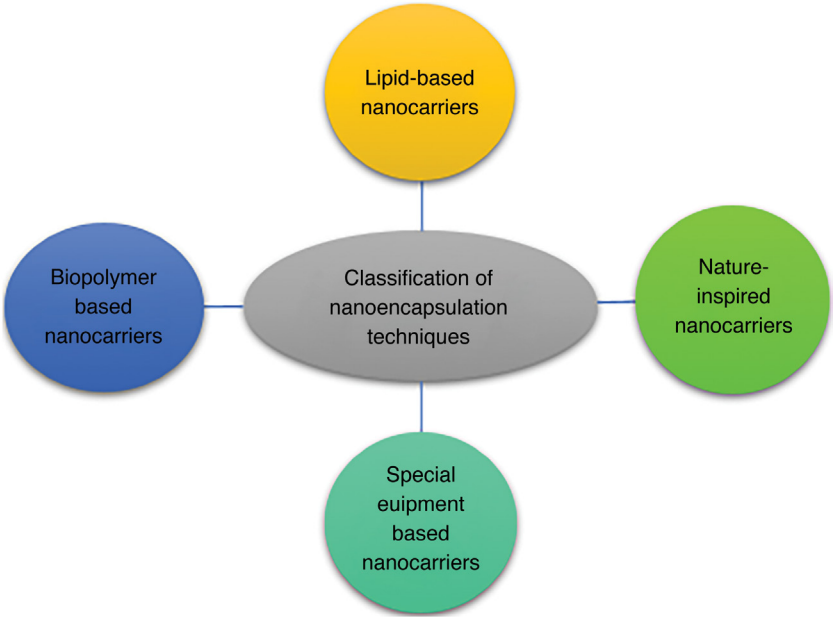


FIGURE 29.2 Classification of nanoencapsulation techniques.

6.1 Lipid-based nanocarriers

Formulation of lipid-based nanocarriers could improve the stability of probiotics in several food products. Nanoencapsulation of probiotics was achieved using different methods such as nanoemulsions, nanostructured phospholipids, and nanolipid carriers (Jafari, 2019). Nanoemulsion technique consists of aqueous and oil phase. The aqueous phase contains probiotics incorporated with proteins or prebiotics, and oil phase enhances the emulsification during the homogenization process. Protein-based nanoemulsions using pea/milk proteins would improve the bioavailability of probiotics (*Enterococcus faecium* and *Bifidobacterium animalis* subsp. *lactis* Bb12) in food products, which reduces the evaporation of food aroma and air induced degradation (Krithika & Preetha, 2019; Lisova, Horáčková, Kováčová, Rada, & Plockova, 2013).

6.2 Nature-inspired nanocarriers

In this method, renewable nanomaterials are used to encapsulate probiotics and considered as a green alternative to synthetic materials. This method includes many advantages like low cost, availability of materials, biodegradability, long-term durability, and nontoxicity. Some nature-inspired nanocarriers, namely, cyclodextrins, caseins, and amylose nanohelices have been used for the encapsulation of probiotic strains to improve their stability in food products (Nedovic, Kalusevic, Manojlovic, Levic, & Bugarski, 2011).

6.3 Special equipment-based nanocarriers

The nanoencapsulation of probiotics is also achieved by special equipment using various techniques like electrospinning and electrospraying. Electrospinning is a cost-effective technique and produces a large volume of nanofibers by applying a high voltage to the polymer solution (Assadpour & Jafari, 2019). This fabrication technique required synthetic polymers like polyvinyl alcohol and polyvinyl pyrrolidine to produce fine nanofibers along with sodium alginate and other prebiotics. Many parameters influence the process of electrospinning such as spinning parameters (applied voltage, the distance between the needle and collector, flow rate, and needle diameter) and solution parameters (polymer concentration, viscosity, and solution conductivity), which affects the fiber production (Haider, Haider, & Kang, 2018). Probiotic bacteria *L. brevis* E25 encapsulated in nanofiber have been used in the production of functional yoghurt and other beverages like apple and orange juices (Mohaisen, Yildirim, Yilmaz, & Durak, 2019). Probiotic strains *L. acidophilus*, *L. plantarum*, and *B. animalis* subsp. *lactis* Bb12 were incorporated into nanofibers using agro-waste waste materials and polyvinyl alcohol through electrospinning technique (Feng et al., 2018; Fung et al., 2011; López-Rubio, Sanchez, Wilkanowicz, Sanz, & Lagaron, 2012). Nanoencapsulated probiotic strains and their performance are given in Table 29.4.

Spray drying is a widely used method to encapsulate sensitive probiotic strains in the food industry. It has many advantages (1) simple process, (2) easy to operate, (3) directly converts into powder, and (4) particle size and shape control. Encapsulation by spray dryer could enhance the targeted delivery and efficacy of probiotics. *L. plantarum* and *Bifidobacterium infantis* encapsulated using chia seed and flaxseed proteins by spray dryer showed effective delivery in the GIT (Gensheimer et al., 2007). Nanoencapsulated *L. casei* exhibits good viability when it is encapsulated with bitter guard juice

TABLE 29.4 Nanoencapsulated probiotics and their performance.

Probiotics	Encapsulation materials	GIT tolerance (%)	Reference
Lactobacillus rhamnosus	Alginate and polyvinyl alcohol	83	Fung et al. (2011)
Bacillus coagulans IBRC-M 10807	Pectin with bacterial cellulose	97.7	Ceylan, Meral, Karakaş, Dertli, and Yilmaz (2018)
Lactobacillus acidophilus	Okara, oil palm trunk, and oil palm frond	90	Khorasani and Shojaosadati (2017)
Lactobacillus plantarum	Polyethylene oxide	2	Heunis, Botes, and Dicks (2010)
Micrococcus luteus	Polyethylene oxide	74	Gensheimer et al. (2007)
Escherichia coli	Polyethylene oxide	1	Gensheimer et al. (2007)

TABLE 29.5 Encapsulation of probiotics by using spray drying technique.

Probiotics	Encapsulation material	Cell survival (%)	Reference
Lactobacillus paracasei subsp. *paracasei*	Taro and rice starch	82-91	Alfaro-Galarza et al. (2020)
Saccharomyces cerevisiae and *Issatchenkia occidentalis*	Maltodextrin and sucrose or sorbitol	80	Suryabhan, Lohith, and Anu-Appaiah (2019)
Lactococcus lactis Gh1	Gum arabic and *Synsepalum dulcificum* (miracle fruit)	80-93	Fazilah et al. (2019)
Lactobacillus casei ATCC 393	Alginate	<90	Dimitrellou et al. (2019)
Bifidobacterium animalis subsp. *lactis* BB-12	Maltodextrin and inulin	79-88	Dias et al. (2018)
Lactobacillus acidophilus	Inulin, hi-maize, and trehalose	90-94	Nunes et al. (2018)
Saccharomyces boulardii, *L. acidophilus*, and *Bifidobacterium bifidum*	Gum arabic and β-cyclodextrin	<90	Arslan-Tontul and Erbas (2017)
Lactobacillus rhamnosus GG	Casein and whey protein	<95	Guerin et al. (2017)
L. rhamnosus	Fructooligosaccharides	<96	Rajam and Anandharamakrishnan (2015)
Saccharomyces cerevisiae var. *boulardii*	Gelatin, whey protein concentrate, modified starch, maltodextrin, pea protein isolate, and gum arabic	84	Arslan, Erbas, Tontul, and Topuz (2015)

using a spray dryer (Kalal et al., 2017). *L. paracasei* subsp. *paracasei* encapsulated with taro and resistance starch exhibits increased survival after electrospraying. *L. rhamnosus* encapsulated with fructooligosaccharides found to have greater survival rate (>96%) after electrospraying. Many probiotic strains like *L. casei* BL23, *Propionibacterium freudenreichii* ITG P20, *L. rhamnosus* GG, *L. acidophilus* La-5, *Bifidobacterium* ssp. *lactis*, *L. plantarum* 299v, and *Saccharomyces cerevisiae* var. *boulardii* have been encapsulated by spray drying technique to produce functional probiotic food products (Assadpour & Jafari, 2019). Probiotic strains encapsulated using spray drying are given in Table 29.5.

6.4 Biopolymer-based nanocarriers

Biopolymers are used as a nutraceutical to protect and deliver the probiotics at specific regions in the gut. These nutraceuticals have different morphology, namely, nanoparticles, nanospheres, nanotubes, and nanogels. Polymers from various sources like milk proteins, plant proteins, animal proteins, and polysaccharides are used for the encapsulation of probiotics (Fung et al., 2011). Biopolymers are divided into three groups based on their nature, namely, polysaccharides (sugars), proteins (amino acids), and nucleic acids (nucleotides), and used as biosurfactant, bioadhesive, and bioflocculant in the food industry. Therefore, encapsulation by biopolymer-based nanocarriers/vehicle could provide better protection to probiotics as well as improved nutritional and sensory properties. This edible coating could also improve the safety and shelf life of ready-to-eat food products. Polysaccharide-based hydrogels also served as an efficient vehicle to encapsulate probiotics strains like *Bifidobacterium breve* and *L. plantarum* using alginate and chitosan particles for effective oral delivery (Bosnea et al., 2017; Li et al., 2017). Probiotic strains encapsulated with chitosan coating could improve the mechanical stability of probiotic starter culture in fermented whey beverages and other dairy products (Zaeim, Sarabi-Jamab, Ghorani, Kadkhodaee, & Tromp, 2017). In addition, polysaccharide nanoparticles as a prebiotic would enhance the antibacterial properties in *L. plantarum* (Hong et al., 2019).

7 Encapsulation of probiotics: insights into industrial applications

Over the past decade, probiotic strains are used for making fermented dairy products. Recently, the uses of probiotics have been extended to nondairy products due to their wide range of health benefits in humans as well as animals. The encapsulation technology could improve the novelty of food products in the market and provides low food processing cost and viable probiotic delivery to GIT (Patil, Chavanke, & Wagh, 2012). Many functional foods have been produced by using probiotics like milk, yoghurts, cheeses, creams, ice creams, meats and meat products, bread and cereal fiber snacks, and juices and other fruit products (Terpou et al., 2019). Many strains have been identified as potential probiotics, but only a few strains sustained in the food market. *Lactobacillus* and *Bifidobacterium* species produce large amount of probiotic food products than *S. cerevisiae*, *E. coli*, and *Bacillus* species. Probiotic strains such as *L. acidophilus*, *L. rhamnosus*, *L. reuteri*,

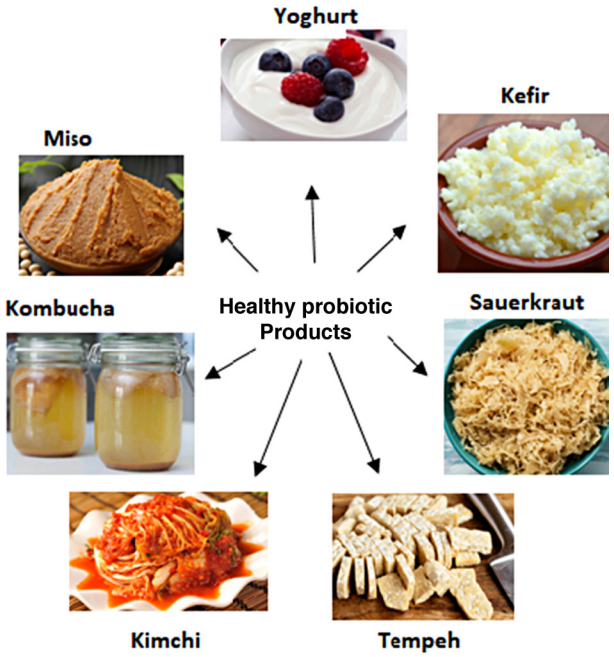


FIGURE 29.3 Commercial probiotic foods.

L. plantarum, *L. fermentum*, *L. brevis B. breve*, *B. infantis*, *B. longum*, *Bifidobacterium lactis*, and *B. bifidum* have been widely used for the industrial applications.

Probiotic-incorporated/encapsulated novel food products have gained more attention in consumers due to their beneficial health effects. The processing technique cannot be the same for all kinds of probiotic foods to retain their stability and viability. Thus, encapsulation could improve the viability of probiotics during food processing and storage.

Probiotic products are used as a dietary supplement in humans and animals. A large number of probiotic foods are produced by dairy and fermented industries. We are consuming many probiotic products in our day-to-day life (Fig. 29.3). The most significant probiotic products are functional beverages that act as an efficient vehicle for probiotics. Also, milk and milk products are considered as an excellent carrier for probiotics due to their properties. Moreover, the storage temperature of milk and milk products is most suitable for probiotic strains. The functional fermented dairy products are mainly composed of fortified milk, or whey protein, which can be utilized as prebiotics by gut microbiome (Song et al., 2019). Probiotic-based milk products reduce the number of gastrointestinal diseases including *Helicobacter pylori* infection, irritable bowel syndrome, and antibiotic-associated diarrhea (Balakrishnan & Floch, 2012; Hosseini, Nikfar, & Abdollahi, 2012).

Encapsulation in plain alginate beads with a combination of other coating materials such as chitosan, starch, pectin, and whey protein--based matrix could improve the viability and stability of probiotics in yoghurt. Hassan, Fadzil, Yusoff, and Khalil (2020) suggest that ice cream and yogurt may serve as ideal probiotic carriers for successful encapsulation. Many probiotic products have been used for various applications in humans and animals (Table 29.6).

Probiotic functional food products are classified into three categories based on their encapsulation materials, namely, (1) dairy-based probiotic products (milk, yogurt, amasi, doogh, ricera, ogi, and bogobe), (2) cereal-based probiotic products (fura, boza, uji, hardaliye, tempoyak, kimchi, and sauerkraut), and (3) fruit- and vegetable-based probiotic products (jiang-gua, tursu, ngari, jeotgal, suan yu, and kitoza). Among these products, Yakult, amasi, kombucha, and doogh are traditional food items that are being produced at industrial scale. Similarly, koumiss, kefir, and sour beer are also used as probiotic food products (Behera & Panda, 2020).

8 Conclusion and future perspectives

The encapsulation technology is considered as a useful tool for delivering probiotics for both dairy and nondairy food products. It ensures the recommended level of probiotics (10^7-10^8 CFU/mL) in food products. Probiotics encapsulation technology (PET) emerged in the 1990s, and tremendous advances have been noted in this field. This technology is being improved constantly, then modified and adapted for practical applications. PET/microencapsulation mainly involves emulsification and extrusion technology. In emulsification, emulsifier or surfactant needs to be added in vegetable oil to promote

TABLE 29.6 Commercial probiotic products and their applications.

Brand/product name	Probiotic strains	Applications/benefits	Web reference
Attune foods	*Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium lactis*	Specialized gluten-free products and clinically proven probiotics bar to balance gut microbiome and improved immune system	www.attunefoods.com
Culturelle	*Lactobacillus rhamnosus* GG	Reducing colic in infants, lowering the risk of colon cancer, to stimulating the immune system	www.culturelle.com
Bio-K + Probiotics	*L. acidophilus*	Used to treat diarrhea, irritable bowel, lactose intolerance, vaginal and urinary tract infections	www.biokplus.com
BioGaia	*Lactobacillus reuteri*	Enforced gut barrier, development of immune system, improved digestion and motility in children	www.biogaia.com
Bifodan	*L. rhamnosus*, *Lactobacillus gasseri*, Lactobacillus *curvatus*, *L. acidophilus*, *Bifidobacterium longum*	Natural defense against oral health problems, improve digestive system, balance immune system, improves intimate health in women	https://bifodan.com
BioCare Copenhagen	Yeast cells and lactic acid bacteria	Specialized ingredients for women's health	www.biocarecph.com
Danone	*Bifidobacterium animalis*	Helps to treat irritable bowel syndrome and inflammatory bowel disease	www.danone.com
Danisco	Consortium of *Bifidobacterium*, *Lactobacillus*, and *Streptococcus thermophilus*	Used as an additive to antibiotics to improve gut health in poultry	www.food.dupont.com
Daflorn	*Lactobacillus bulgaricus*	Beverages	www.daflorn.com
Yakult	*Lactobacillus paracasei*	Probiotic solutions	www.yakult.co.in
Winclove Probiotics	Mixture of *Lactobacillus* sp.	Restored microbiome, women's health, and upper respiratory tract infections	www.wincloveprobiotics.com
Probi	*Lactobacillus plantarum*, *L. paracasei*	Infant probiotic products	https://probi.com
Nestle	*Lactobacillus fermentum*, *L. acidophilus*, *L. paracasei*	Specialized probiotic ingredients for breast feeding mothers	www.nestle.in
Lallemand	*Saccharomyces cerevisiae* var. *boulardii*	Used as animal feed for poultry, ruminants, and horse	www.lallemand.com

capsule. This emulsifier or residual oil or surfactant may be toxic to probiotic cells and can interact with food components. Also, residual oil is detrimental to the texture and organoleptic properties of a food product. Moreover, it is a more expensive method and needs additional raw material such as oil/emulsifier for stabilization of the capsules. Extrusion technology seems to be better for probiotics encapsulation in terms of cost, handling conditions, and safety requirements.

In extrusion, many techniques have been used to encapsulate probiotics, but the layer-by-layer approach stands out. In layer-by-layer approach, probiotics are initially mixed with efficient core material (alginate and oligosaccharides) and then coated with one or more efficient matrices like chitosan and whey protein. Hence, food matrices are considered as an effective carrier for microencapsulation, and they ensure the recommended viability during food processing and storage. Also, it enhances the targeted delivery of probiotics in GIT. However, many challenges still exist in PET such as processing cost, development of equipment, procedures, and selection of nontoxic and effective materials that could be adapted to the pH of the digestive tract to determine the mechanisms of probiotic release from microcapsules or beads and safety assessment of microcapsules using in vitro and in vivo assays.

Nanoencapsulation is an emerging encapsulation technology. Use of electrical devices and advances in nanotechnology may lead to more sophisticated developments in encapsulation. Encapsulation using special equipment provides excellent protection to probiotics. Hence, electrospinning/spray dyer can be chosen as a potential technique to incorporate probiotics into food products. So far only a few strains have been encapsulated using this technique. Further research is needed to develop and modify the equipment design, encapsulation process, and materials. Nanoencapsulation can play an essential role in the future, which can overcome the problem of maintaining probiotic viability, stability, and delivery.

Acknowledgment

Authors are thankful to Vellore Institute of Technology (VIT) for their support throughout the study.

References

Abd El-Salam, M. H., & El-Shibiny, S. (2012). Formation and potential uses of milk proteins as nano delivery vehicles for nutraceuticals: A review. *International Journal of Dairy Technology*, *65*(1), 13–21.

Abdelkader, H., Hussain, S. A., & Abdullah, N. (2018). Review on micro-encapsulation with Chitosan for pharmaceuticals applications. *MOJ Current Research & Reviews*, *1*(2), 77–84.

Afzaal, M., Saeed, F., Arshad, M. U., Nadeem, M. T., Saeed, M., & Tufail, T. (2019). The effect of encapsulation on the stability of probiotic bacteria in ice cream and simulated gastrointestinal conditions. *Probiotics and Antimicrobial Proteins*, *11*(4), 1348–1354.

Agudelo, J., Cano, A., González-Martínez, C., & Chiralt, A. (2017). Disaccharide incorporation to improve survival during storage of spray dried *Lactobacillus rhamnosus* in whey protein-maltodextrin carriers. *Journal of Functional Foods*, *37*, 416–423.

Alehosseini, E., & Jafari, S. M. (2019). Micro/nano-encapsulated phase change materials (PCMs) as emerging materials for the food industry. *Trends in Food Science and Technology*, *91*, 116–128.

Alfaro-Galarza, O., López-Villegas, E. O., Rivero-Perez, N., Tapia-Maruri, D., Jiménez-Aparicio, A. R., Palma-Rodríguez, H. M., et al. (2020). Protective effects of the use of taro and rice starch as wall material on the viability of encapsulated *Lactobacillus paracasei* subsp. *paracasei*. *LWT*, *117*, 108686.

Ali, S. A., Singh, P., Tomar, S. K., Mohanty, A. K., & Behare, P. (2020). Proteomics fingerprints of systemic mechanisms of adaptation to bile in *Lactobacillus fermentum*. *Journal of Proteomics*, *213*, 103600.

Alli, S. M. A. (2013). Development of product containing microencapsulated probiotics: An update on issues. *Journal of Drug Delivery and Therapeutics*, *3*(5), 121–131.

Alvarez-Olmos, M. I., & Oberhelman, R. A. (2001). Probiotic agents and infectious diseases: A modern perspective on a traditional therapy. *Clinical Infectious Diseases*, *32*(11), 1567–1576.

Amund, O. D. (2016). Exploring the relationship between exposure to technological and gastrointestinal stress and probiotic functional properties of lactobacilli and bifidobacteria. *Canadian Journal of Microbiology*, *62*(9), 715–725.

Archacka, M., Białas, W., Dembczyński, R., Olejnik, A., Sip, A., Szymanowska, D., et al. (2019). Method of preservation and type of protective agent strongly influence probiotic properties of *Lactococcus lactis*: A complete process of probiotic preparation manufacture and use. *Food Chemistry*, *274*, 733–742.

Arslan, S., Erbas, M., Tontul, I., & Topuz, A. (2015). Microencapsulation of probiotic *Saccharomyces cerevisiae* var. *boulardii* with different wall materials by spray drying. *LWT-Food Science and Technology*, *63*(1), 685–690.

Arslan-Tontul, S., & Erbas, M. (2017). Single and double layered microencapsulation of probiotics by spray drying and spray chilling. *LWT-Food Science and Technology*, *81*, 160–169.

Ashwar, B. A., Gani, A., Gani, A., Shah, A., & Masoodi, F. A. (2018). Production of RS4 from rice starch and its utilization as an encapsulating agent for targeted delivery of probiotics. *Food Chemistry*, *239*, 287–294.

Assadpour, E., & Jafari, S. M. (2019). An overview of specialized equipment for nanoencapsulation of food ingredients. In *Nanoencapsulation of food ingredients by specialized equipment* (pp. 1–30). United States: Academic Press.

Balakrishnan, M., & Floch, M. H. (2012). Prebiotics, probiotics and digestive health. *Current Opinion in Clinical Nutrition and Metabolic Care*, *15*(6), 580–585.

Begum, R., Najeeb, J., Ahmad, G., Wu, W., Irfan, A., Al-Sehemi, A. G., et al. (2018). Synthesis and characterization of poly (N-isopropylmethacrylamide-co-acrylic acid) microgels for in situ fabrication and stabilization of silver nanoparticles for catalytic reduction of o-nitroaniline in aqueous medium. *Reactive & Functional Polymers*, *132*, 89–97.

Behera, S. S., & Panda, S. K. (2020). Ethnic and industrial probiotic foods and beverages: Efficacy and acceptance. *Current Opinion in Food Science*, *32*, 29–36.

Bermudez-Brito, M., Plaza-Díaz, J., Munoz-Quezada, S., Gomez-Llorente, C., & Gil, A. (2012). Probiotic mechanisms of action. *Annals of Nutrition and Metabolism*, *61*(2), 160–174.

Bhadoria, P. B. S., & Mahapatra, S. C. (2011). Prospects, technological aspects and limitations of probiotics - A worldwide review. *European Journal of Nutrition & Food Safety*, *1*(2), 23–42.

Boricha, A. A., Shekh, S. L., Pithva, S. P., Ambalam, P. S., & Vyas, B. R. M. (2019). In vitro evaluation of probiotic properties of *Lactobacillus* species of food and human origin. *LWT*, *106*, 201–208.

Bosnea, L. A., Moschakis, T., Nigam, P. S., & Biliaderis, C. G. (2017). Growth adaptation of probiotics in biopolymer-based coacervate structures to enhance cell viability. *LWT*, *77*, 282–289.

Bustos, A. Y., de Valdez, G. F., Fadda, S., & Taranto, M. P. (2018). New insights into bacterial bile resistance mechanisms: The role of bile salt hydrolase and its impact on human health. *Food Research International*, *112*, 250–262.

Ceylan, Z., Meral, R., Karakaş, C. Y., Dertli, E., & Yilmaz, M. T. (2018). A novel strategy for probiotic bacteria: Ensuring microbial stability of fish fillets using characterized probiotic bacteria-loaded nanofibers. *Innovative Food Science and Emerging Technologies*, *48*, 212–218.

Champagne, C. P., da Cruz, A. G., & Daga, M. (2018). Strategies to improve the functionality of probiotics in supplements and foods. *Current Opinion in Food Science*, *22*, 160–166.

Cheng, D., Song, J., Xie, M., & Song, D. (2019). The bidirectional relationship between host physiology and microbiota and health benefits of probiotics: A review. *Trends in Food Science and Technology*, *91*, 426–435.

Chew, S., Tan, C., Pui, L., Chong, P., Gunasekaran, B., & Nyam, K. (2019). Encapsulation technologies: A tool for functional foods development. *International Journal of Innovative Technology and Exploring Engineering*, *8*(5S), 154–162.

Chua, K. J., Kwok, W. C., Aggarwal, N., Sun, T., & Chang, M. W. (2017). Designer probiotics for the prevention and treatment of human diseases. *Current Opinion in Chemical Biology*, *40*, 8–16.

Chugh, B., & Kamal-Eldin, A. (2020). Bioactive compounds produced by probiotics in food products. *Current Opinion in Food Science*, *32*, 76–82.

Coqueiro, A. Y., Raizel, R., Bonvini, A., Tirapegui, J., & Rogero, M. M. (2019). Probiotics for inflammatory bowel diseases: A promising adjuvant treatment. *International Journal of Food Sciences and Nutrition*, *70*(1), 20–29.

da Silva, E. M., Penelas, A. G., Simmer, F. S., de Paiva, R. V., Moreira e Silva, V. L., & Poskus, L. T. (2018). Can the use of a warm-air stream for solvent evaporation lead to a dangerous temperature increase during dentin hybridization? *Journal of Adhesive Dentistry*, *20*(4), 335–340.

de Araújo Etchepare, M., Nunes, G. L., Nicoloso, B. R., Barin, J. S., Flores, E. M. M., de Oliveira Mello, R., et al. (2020). Improvement of the viability of encapsulated probiotics using whey proteins. *LWT*, *117*, 108601.

de Moraes, G. M. D., dos Santos, K. M. O., de Barcelos, S. C., Lopes, S. A., & do Egito, A. S. (2018). Potentially probiotic goat cheese produced with autochthonous adjunct culture of *Lactobacillus mucosae*: Microbiological, physicochemical and sensory attributes. *LWT*, *94*, 57–63.

de Oca-Ávalos, J. M. M., Candal, R. J., & Herrera, M. L. (2017). Nanoemulsions: Stability and physical properties. *Current Opinion in Food Science*, *16*, 1–6.

de Oliveira Ribeiro, A. P., dos Santos Gomes, F., dos Santos, K. M. O., da Matta, V. M., de Araujo Santiago, M. C. P., Conte, C., et al. (2020). Development of a probiotic non-fermented blend beverage with juçara fruit: Effect of the matrix on probiotic viability and survival to the gastrointestinal tract. *LWT*, *118*, 108756.

De Prisco, A., & Mauriello, G. (2016). Probiotication of foods: A focus on microencapsulation tool. *Trends in Food Science and Technology*, *48*, 27–39.

Dehkordi, S. S., Alemzadeh, I., Vaziri, A. S., & Vossoughi, A. (2020). Optimization of Alginate-Whey protein isolate microcapsules for survivability and release behavior of probiotic bacteria. *Applied Biochemistry and Biotechnology*, *190*(1), 182–196.

den Besten, G., van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D. J., & Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research*, *54*(9), 2325–2340.

Diana, A., & Purwanti, T. (2019). Utilization of *Lactobacillus acidophilus* FNcc-0051 microencapsulation: Potential benefit of giving combination of sodium alginate and gelatin to attributes and role of probiotic against *Staphylococcus aureus*. *Indian Journal of Public Health Research & Development*, *10*(9), 1410–1415.

Dianawati, D., Mishra, V., & Shah, N. P. (2016). Survival of microencapsulated probiotic bacteria after processing and during storage: A review. *Critical Reviews in Food Science and Nutrition*, *56*(10), 1685–1716.

Dias, C. O., Opuski, S., Pinto, S. S., Cristina, F., Santana, D. O., Müller, C. M. O., et al. (2018). Development and physicochemical characterization of microencapsulated *Bifidobacteria* in passion fruit juice: A functional non-dairy product for probiotic delivery. *Food Bioscience*, *24*, 26–36.

Dimitrellou, D., Kandylis, P., Lević, S., Petrović, T., Ivanović, S., Nedović, V., et al. (2019). Encapsulation of *Lactobacillus casei* ATCC 393 in alginate capsules for probiotic fermented milk production. *LWT-Food Science and Technology*, *116*, 108501.

Dinkçi, N., Akdeniz, V., & Akalin, A. S. (2019). Survival of probiotics in functional foods during shelf life. In *Food quality and shelf life* (pp. 201–233). United States: Academic Press.

Dlamini, Z. C., Langa, R. L., Aiyegoro, O. A., & Okoh, A. I. (2019). Safety evaluation and colonisation abilities of four lactic acid bacteria as future probiotics. *Probiotics and Antimicrobial Proteins*, *11*(2), 397–402.

Donsì, F., Sessa, M., & Ferrari, G. (2019). Encapsulation of bioactive compounds. *Applications of Encapsulation and Controlled Release*, *405*.

Ebeid, T. A., Fathi, M. M., Al-Homidan, I., Ibrahim, Z. H., & Al-Sagan, A. A. (2019). Effect of dietary probiotics and stocking density on carcass traits, meat quality, microbial populations and ileal histomorphology in broilers under hot-climate conditions. *Animal Production Science*, *59*(9), 1711–1719.

Eckert, C., Agnol, W. D., Dallé, D., Serpa, V. G., Maciel, M. J., Lehn, D. N., et al. (2018). Development of alginate-pectin microparticles with dairy whey using vibration technology: Effects of matrix composition on the protection of *Lactobacillus* spp. from adverse conditions. *Food Research International*, *113*, 65–73.

El Enshasy, H., Malik, K., Malek, R. A., Othman, N. Z., Elsayed, E. A., & Wadaan, M. (2016). Anaerobic probiotics: The key microbes for human health. In *Anaerobes in biotechnology* (pp. 397–431). Cham: Springer.

Er, S., Tosun, A. İ., Berk, G. A., & Kivanc, M. (2019). Anticandidal activities of lactic acid bacteria isolated from the vagina. *Turkish Journal of Medical Sciences*, *49*(1), 375–383.

Eshrati, M., Amadei, F., Van de Wiele, T., Veschgini, M., Kaufmann, S., & Tanaka, M. (2018). Biopolymer-based minimal formulations boost viability and metabolic functionality of probiotics *Lactobacillus rhamnosus* GG through gastrointestinal passage. *Langmuir*, *34*(37), 11167–11175.

Ezhilarasi, P. N., Karthik, P., Chhanwal, N., & Anandharamakrishnan, C. (2013). Nanoencapsulation techniques for food bioactive components: A review. *Food and Bioprocess Technology*, *6*(3), 628–647.

Fang, Z., & Bhandari, B. (2010). Encapsulation of polyphenols - A review. *Trends in Food Science and Technology*, *21*(10), 510–523.

Farbo, M. G., Urgeghe, P. P., Fiori, S., Marceddu, S., Jaoua, S., & Migheli, Q. (2016). Adsorption of ochratoxin A from grape juice by yeast cells immobilised in calcium alginate beads. *International Journal of Food Microbiology*, *217*, 29–34.

Fazilah, N. F., Hamidon, N. H., Ariff, A. B., Khayat, M. E., Wasoh, H., & Halim, M. (2019). Microencapsulation of *Lactococcus lactis* Gh1 with Gum Arabic and *Synsepalum dulcificum* via Spray Drying for Potential Inclusion in Functional Yogurt. *Molecules*, *24*(7), 1422.

Feng, G., Flanagan, B. M., Williams, B. A., Mikkelsen, D., Yu, W., & Gidley, M. J. (2018). Extracellular depolymerisation triggers fermentation of tamarind xyloglucan and wheat arabinoxylan by a porcine faecal inoculum. *Carbohydrate Polymers*, *201*, 575–582.

Feng, K., Huang, R. M., Wu, R. Q., Wei, Y. S., Zong, M. H., Linhardt, R. J., et al. (2020). A novel route for double-layered encapsulation of probiotics with improved viability under adverse conditions. *Food Chemistry*, *310*, 125977.

Fontana, A., Falasconi, I., Molinari, P., Treu, L., Basile, A., Vezzi, A., et al. (2019). Genomic comparison of *Lactobacillus helveticus* strains highlights probiotic potentials. *Frontiers in Microbiology*, *10*, 1380.

Fung, W. Y., Yuen, K. H., & Liong, M. T. (2011). Agrowaste-based nanofibers as a probiotic encapsulant: Fabrication and characterization. *Journal of Agricultural and Food Chemistry*, *59*(15), 8140–8147.

Gandomi, H., Abbaszadeh, S., Misaghi, A., Bokaie, S., & Noori, N. (2016). Effect of chitosan-alginate encapsulation with inulin on survival of *Lactobacillus rhamnosus* GG during apple juice storage and under simulated gastrointestinal conditions. *LWT-Food Science and Technology*, *69*, 365–371.

Gensheimer, M., Becker, M., Brandis-Heep, A., Wendorff, J. H., Thauer, R. K., & Greiner, A. (2007). Novel biohybrid materials by electrospinning: Nanofibers of poly (ethylene oxide) and living bacteria. *Advanced Materials*, *19*(18), 2480–2482.

González-Ferrero, C., Irache, J. M., Marín-Calvo, B., Ortiz-Romero, L., Virto-Resano, R., & González-Navarro, C. J. (2020). Encapsulation of probiotics in soybean protein-based microparticles preserves viable cell concentration in foods all along the production and storage processes. *Journal of Microencapsulation*, *37*(3), 1–34.

Gopinath, V., Saravanan, S., Al-Maleki, A. R., Ramesh, M., & Vadivelu, J. (2018). A review of natural polysaccharides for drug delivery applications: Special focus on cellulose, starch and glycogen. *Biomedicine & Pharmacotherapy*, *107*, 96–108.

Górska, A., Przystupski, D., Niemczura, M. J., & Kulbacka, J. (2019). Probiotic bacteria: A promising tool in cancer prevention and therapy. *Current Microbiology*, *76*, 1–11.

Gu, M., Zhang, Z., Pan, C., Goulette, T. R., Zhang, R., Hendricks, G., et al. (2019). Encapsulation of *Bifidobacterium pseudocatenulatum* G7 in gastroprotective microgels: Improvement of the bacterial viability under simulated gastrointestinal conditions. *Food Hydrocolloid*, *91*, 283–289.

Guerin, J., Petit, J., Burgain, J., Borges, F., Bhandari, B., Perroud, C., et al. (2017). *Lactobacillus rhamnosus* GG encapsulation by spray-drying: Milk proteins clotting control to produce innovative matrices. *Journal of Food Engineering*, *193*, 10–19.

Gwak, H. J., Lee, J. H., Kim, T. W., Choi, H. J., Jang, J. Y., Lee, S. I., et al. (2015). Protective effect of soy powder and microencapsulation on freeze-dried *Lactobacillus brevis* WK12 and *Lactococcus lactis* WK11 during storage. *Food Science and Biotechnology*, *24*(6), 2155–2160.

Hadzieva, J., Mladenovska, K., Simonoska Crcarevska, M., Glavaš Dodov, M., Dimchevska, S., Geškovski, N., et al. (2017). *Lactobacillus casei* encapsulated in soy protein isolate and alginate microparticles prepared by spray drying. *Food Technology and Biotechnology*, *55*(2), 173–186.

Haghshenas, B., Abdullah, N., Nami, Y., Radiah, D., Rosli, R., & Yari Khosroushahi, A. (2015). Microencapsulation of probiotic bacteria *Lactobacillus plantarum* 15 HN using alginate-psyllium-fenugreek polymeric blends. *Journal of Applied Microbiology*, *118*(4), 1048–1057.

Haider, A., Haider, S., & Kang, I. K. (2018). A comprehensive review summarizing the effect of electrospinning parameters and potential applications of nanofibers in biomedical and biotechnology. *Arabian Journal of Chemistry*, *11*(8), 1165–1188.

Hammam, A. R., & Ahmed, M. S. (2019). Technological aspects, health benefits, and sensory properties of probiotic cheese. *SN Applied Sciences*, *1*(9), 1113.

Hassan, S. A. A., Hung, P. K. J., & Sarbini, S. R. (2019). Evaluation of bacteria isolated from the gastrointestinal tract of termites (*Coptotermes curvignathus*) as potential probiotics. *Journal of Agriculture Food and Development*, *5*, 63–70.

Hassan, S. S., Fadzil, I. N. A., Yusoff, A., & Khalil, K. A. (2020). A review on microencapsulation in improving probiotic stability for beverages application. *Science Letters*, *14*, 1.

Hati, S., Das, S., & Mandal, S. (2019). Technological advancement of functional fermented dairy beverages. In *Engineering tools in the beverage industry* (pp. 101–136). United Kingdom: Woodhead Publishing.

Heunis, T. D. J., Botes, M., & Dicks, L. M. T. (2010). Encapsulation of *Lactobacillus plantarum* 423 and its bacteriocin in nanofibers. *Probiotics and Antimicrobial Proteins*, *2*(1), 46–51.

Hols, P., Ledesma-García, L., Gabant, P., & Mignolet, J. (2019). Mobilization of microbiota commensals and their bacteriocins for therapeutics. *Trends in Microbiology*, *27*(8), 690–702.

Hong, L., Kim, W. S., Lee, S. M., Kang, S. K., Choi, Y. J., & Cho, C. S. (2019). Pullulan nanoparticles as prebiotics enhanced anti-bacterial properties of *Lactobacillus plantarum* through an induction of mild stress in probiotics. *Frontiers in Microbiology*, *10*, 142.

Horáčková, Š., Plocková, M., & Demnerová, K. (2018). Importance of microbial defence systems to bile salts and mechanisms of serum cholesterol reduction. *Biotechnology Advances*, *36*(3), 682–690.

Hosseini, A., Nikfar, S., & Abdollahi, M. (2012). Probiotics use to treat irritable bowel syndrome. *Expert Opinion on Biological Therapy*, *12*(10), 1323–1334.

Hu, P. L., Yuan, Y. H., Yue, T. L., & Guo, C. F. (2018). Bile acid patterns in commercially available oxgall powders used for the evaluation of the bile tolerance ability of potential probiotics. *PLoS One*, *13*(3), e0192964.

Hugues-Ayala, A. M., Sarabia-Sainz, J. A. I., González-Rios, H., Vázquez-Moreno, L., & Montfort, G. R. C. (2020). Airbrush encapsulation of *Lactobacillus rhamnosus* GG in dry microbeads of alginate coated with regular buttermilk proteins. *LWT*, *117*, 108639.

Huq, T., Fraschini, C., Khan, A., Riedl, B., Bouchard, J., & Lacroix, M. (2017). Alginate based nanocomposite for microencapsulation of probiotic: Effect of cellulose nanocrystal (CNC) and lecithin. *Carbohydrate Polymers*, *168*, 61–69.

Iannitti, T., & Palmieri, B. (2010). Therapeutical use of probiotic formulations in clinical practice. *Clinical Nutrition*, *29*(6), 701–725.

Iqbal, R., Zahoor, T., Huma, N., Jamil, A., & Ünlü, G. (2019). In-vitro GIT tolerance of microencapsulated *Bifidobacterium bifidum* ATCC 35914 using polysaccharide-protein matrix. *Probiotics and Antimicrobial Proteins*, *11*(3), 830–839.

Jafari, S. (2019). In *Biopolymer nanostructures for food encapsulation purposes: Volume 1 in the nanoencapsulation in the food industry series. Vol. 1*. United States: Academic Press.

Judkins, T. C., Archer, D. L., Kramer, D. C., & Solch, R. J. (2020). Probiotics, nutrition, and the small intestine. *Current Gastroenterology Reports*, *22*(1), 2.

Kalal, A. Y., Hiregoudar, S., Nidoni, U., Ramachandra, C. T., Naik, N., & Roopabai, R. S. (2017). Nanoencapsulation of *Lactobacillus casei* in bitter gourd juice using spray drying. *Advanced in Bioresource*, *8*(6), .

Kerry, R. G., Patra, J. K., Gouda, S., Park, Y., Shin, H. S., & Das, G. (2018). Benefaction of probiotics for human health: A review. *Journal of Food and Drug Analysis*, *26*(3), 927–939.

Khan, P. F. K. A., Rohit, P., Pave, A. C., Sanap, M. D., Guajarati, N. A., Rane, B. R., et al. (2015). A review on microencapsulation. *Pharmaceutical Sciences*, *6*(2), .

Khorasani, A. C., & Shojaosadati, S. A. (2017). Starch-and carboxymethylcellulose-coated bacterial nanocellulose-pectin bionanocomposite as novel protective prebiotic matrices. *Food Hydrocolloid*, *63*, 273–285.

Kim, M., Nam, D. G., Im, P., Choe, J. S., & Choi, A. J. (2020). Optimal conditions for the encapsulation of *Weissella cibaria* JW15 using alginate and chicory root and evaluation of capsule stability in a simulated gastrointestinal system. *Journal of Food Science*, *85*(2), 394–403.

Krasaekoopt, W., & Kitsawad, K. (2010). Sensory characteristics and consumer acceptance of fruit juice containing probiotics beads in Thailand. *AU Journal of Technolog*, *14*(1), 33–38.

Krithika, B., & Preetha, R. (2019). Formulation of protein based inulin incorporated synbiotic nanoemulsion for enhanced stability of probiotic. *Materials Research Express*, *6*(11), 114003.

Krunić, T. Ž., Obradović, N. S., & Rakin, M. B. (2019). Application of whey protein and whey protein hydrolysate as protein based carrier for probiotic starter culture. *Food Chemistry*, *293*, 74–82.

Kumar, K. S., Tejbe, S., Banu, S., Lakshmi, P. N., & Bhowmik, D. (2013). Microencapsulation technology. *Indian Journal of Research in Pharmacy and Biotechnology*, *1*(3), 324.

Kumar, S., Pandey, R. K., Negi, H., Sharma, P., Pandey, P., Pandey, Y., et al. (2018). Role of probiotics in health improvement: Adaptations, advantages and their uses. *Asian Food Science*, *2*(1), 1–15.

Kwiecień, I., & Kwiecień, M. (2018). Application of polysaccharide-based hydrogels as probiotic delivery systems. *Gels*, *4*(2), 47.

Li, W., Zhu, Y., Ye, F., Li, B., Luo, X., & Liu, S. (2017). Probiotics in cellulose houses: Enhanced viability and targeted delivery of *Lactobacillus plantarum*. *Food Hydrocolloid*, *62*, 66–72.

Liao, H., Liu, S., Wang, H., Su, H., & Liu, Z. (2019). Enhanced antifungal activity of bovine lactoferrin-producing probiotic *Lactobacillus casei* in the murine model of vulvovaginal candidiasis. *BMC Microbiology*, *19*(1), 7.

Lisova, I., Horáčková, Š., Kováčová, R., Rada, V., & Plockova, M. (2013). Emulsion encapsulation of *Bifidobacterium animalis* subsp. *lactis* BB12 with the addition of lecithin. *Czech Journal of Food Science*, *31*(3), 270–274.

Liu, H., Cui, S. W., Chen, M., Li, Y., Liang, R., Xu, F., et al. (2019). Protective approaches and mechanisms of microencapsulation to the survival of probiotic bacteria during processing, storage and gastrointestinal digestion: A review. *Critical Reviews in Food Science and Nutrition*, *59*(17), 2863–2878.

Lopes, S., Bueno, L., Aguiar Júnior, F. D., & Finkler, C. (2017). Preparation and characterization of alginate and gelatin microcapsules containing *Lactobacillus rhamnosus*. *Anais da Academia Brasileira de Ciências*, *89*(3), 1601–1613.

López-Rubio, A., Sanchez, E., Wilkanowicz, S., Sanz, Y., & Lagaron, J. M. (2012). Electrospinning as a useful technique for the encapsulation of living *bifidobacteria* in food hydrocolloids. *Food Hydrocolloid*, *28*(1), 159–167.

Luo, M., Hu, M., Feng, X., XiaoLi, W., Dong, D., & Wang, W. (2020). Preventive effect of *Lactobacillus reuteri* on melanoma. *Biomedicine & Pharmacotherapy*, *126*, 109929.

Maresca, D., Zotta, T., & Mauriello, G. (2018). Adaptation to aerobic environment of *Lactobacillus johnsonii/gasseri* strains. *Frontiers in Microbiology*, *9*, 157.

Martín, M. J., Lara-Villoslada, F., Ruiz, M. A., & Morales, M. E. (2015). Microencapsulation of bacteria: A review of different technologies and their impact on the probiotic effects. *Innovative Food Science and Emerging Technologies*, *27*, 15–25.

Masuda, M., Ide, M., Utsumi, H., Niiro, T., Shimamura, Y., & Murata, M. (2012). Production potency of folate, vitamin B12, and thiamine by lactic acid bacteria isolated from Japanese pickles. *Bioscience, Biotechnology, and Biochemistry*, *76*(11), 2061–2067.

Min, M., Bunt, C. R., Mason, S. L., & Hussain, M. A. (2019). Non-dairy probiotic food products: An emerging group of functional foods. *Critical Reviews in Food Science and Nutrition*, *59*(16), 2626–2641.

Mishra, P., Nayak, B., & Dey, R. K. (2016). PEGylation in anti-cancer therapy: An overview. *Asian Journal of Pharmaceutical Sciences*, *11*(3), 337–348.

Moghanjougi, Z. M., Bari, M. R., Khaledabad, M. A., Almasi, H., & Amiri, S. (2020). Bio-preservation of white brined cheese (Feta) by using probiotic bacteria immobilized in bacterial cellulose: Optimization by response surface method and characterization. *LWT*, *117*, 108603.

Mohaisen, M. J. M., Yildirim, R. M., Yilmaz, M. T., & Durak, M. Z. (2019). Production of functional yogurt drink, apple and orange juice using nano-encapsulated *L. brevis* within sodium alginate-based biopolymers. *Science of Advanced Materials*, *11*(12), 1788–1797.

Molska, M., & Reguła, J. (2019). Potential mechanisms of probiotics action in the prevention and treatment of colorectal cancer. *Nutrients*, *11*(10), 2453.

Mukherjee, A., Verma, J. P., Gaurav, A. K., Chouhan, G. K., Patel, J. S., & Hesham, A. E. L. (2020). Yeast a potential bio-agent: Future for plant growth and postharvest disease management for sustainable agriculture. *Applied Microbiology and Biotechnology*, *104*(4), 1–14.

Mushtaq, M., Gani, A., & Masoodi, F. A. (2019). Himalayan cheese (Kalari/Kradi) fermented with different probiotic strains: In vitro investigation of nutraceutical properties. *LWT*, *104*, 53–60.

Nazir, Y., Hussain, S. A., Abdul Hamid, A., & Song, Y. (2018). Probiotics and their potential preventive and therapeutic role for cancer, high serum cholesterol, and allergic and HIV diseases. *BioMed Research International*, *2018*.

Nedovic, V., Kalusevic, A., Manojlovic, V., Levic, S., & Bugarski, B. (2011). An overview of encapsulation technologies for food applications. *Procedia Food Science*, *1*, 1806–1815.

Nelson, K. D., Crow, B. B., Griffin, N. B., Seifert, J., Sood, P. R., Patel, A. P., et al. (2019). In *U. S. Patent No. 10,363,281. U.S.*. Washington, DC: Patent and Trademark Office.

Ningtyas, D. W., Bhandari, B., Bansal, N., & Prakash, S. (2019). The viability of probiotic *Lactobacillus rhamnosus* (non-encapsulated and encapsulated) in functional reduced-fat cream cheese and its textural properties during storage. *Food Control*, *100*, 8–16.

Nunes, G. L., de Araújo Etchepare, M., Cichoski, A. J., Zepka, L. Q., Lopes, E. J., Barin, J. S., et al. (2018). Inulin, hi-maize, and trehalose as thermal protectants for increasing viability of *Lactobacillus acidophilus* encapsulated by spray drying. *LWT*, *89*, 128–133.

Obradović, N. S., Krunić, T. Ž., Trifković, K. T., Bulatović, M. L., Rakin, M. P., Rakin, M. B., et al. (2015). Influence of chitosan coating on mechanical stability of biopolymer carriers with probiotic starter culture in fermented whey beverages. *International Journal of Polymer Science*, *2015*.

Ozdal, T., Yolci-Omeroglu, P., & Tamer, E. C. (2020). Role of encapsulation in functional beverages. In *Biotechnological progress and beverage consumption* (pp. 195–232). United States: Academic Press.

Pandey, K. R., Naik, S. R., & Vakil, B. V. (2015). Probiotics, prebiotics and synbiotics-a review. *Journal of Food Science and Technology*, *52*(12), 7577–7587.

Panghal, A., Jaglan, S., Sindhu, N., Anshid, V., Charan, M. V. S., Surendran, V., et al. (2019). Microencapsulation for delivery of probiotic bacteria. In *Nanobiotechnology in bioformulations* (pp. 135–160). Cham: Springer.

Patel, A., Shah, N., & Prajapati, J. B. (2013). Biosynthesis of vitamins and enzymes in fermented foods by lactic acid bacteria and related genera-A promising approach. *Croatian Journal of Food Science and Technology*, *5*(2), 85–91.

Patil, P., Chavanke, D., & Wagh, M. (2012). A review on ionotropic gelation method: Novel approach for controlled gastroretentive gelispheres. *International Journal of Pharmacy and Pharmaceutical Sciences*, *4*(4), 27–32.

Pavli, F., Tassou, C., Nychas, G. J. E., & Chorianopoulos, N. (2018). Probiotic incorporation in edible films and coatings: Bioactive solution for functional foods. *International Journal of Molecular Sciences*, *19*(1), 150.

Pech-Canul, A. D. L. C., Ortega, D., García-Triana, A., & González-Silva, N. (2020). A brief review of edible coating materials for the microencapsulation of probiotics. *Coatings*, *10*(3), 197.

Pereira, C. M., Marques, M. F., Hatano, M. K., & Castro, I. A. D. (2010). Effect of the partial substitution of soy proteins by highly methyl-esterified pectin on chemical and sensory characteristics of sausages. *Food Science and Technology International*, *16*(5), 401–407.

Phoem, A. N., Chanthachum, S., & Voravuthikunchai, S. P. (2015). Applications of microencapsulated *Bifidobacterium longum* with *Eleutherine americana* in fresh milk tofu and pineapple juice. *Nutrients*, *7*(4), 2469–2484.

Pitigraisorn, P., Srichaisupakit, K., Wongpadungkiat, N., & Wongsasulak, S. (2017). Encapsulation of *Lactobacillus acidophilus* in moist-heat-resistant multilayered microcapsules. *Journal of Food Engineering*, *192*, 11–18.

Piwowarek, K., Lipińska, E., Hać-Szymańczuk, E., Kieliszek, M., & Ścibisz, I. (2018). *Propionibacterium* spp.-Source of propionic acid, vitamin B12, and other metabolites important for the industry. *Applied Microbiology and Biotechnology*, *102*(2), 515–538.

Pop, O. L., Pop, C. R., Dufrechou, M., Vodnar, D. C., Socaci, S. A., Dulf, F. V., et al. (2020). Edible films and coatings functionalization by probiotic incorporation: A review. *Polymer*, *12*(1), 12.

Pradeep Prasanna, P. H., & Charalampopoulos, D. (2019). Encapsulation in an alginate-goats' milk-inulin matrix improves survival of probiotic *Bifidobacterium* in simulated gastrointestinal conditions and goats' milk yoghurt. *International Journal of Dairy Technology*, *72*(1), 132–141.

Prasanna, P. H. P., & Charalampopoulos, D. (2018). Encapsulation of *Bifidobacterium longum* in alginate-dairy matrices and survival in simulated gastrointestinal conditions, refrigeration, cow milk and goat milk. *Food Bioscience*, *21*, 72–79.

Qi, X., Simsek, S., Ohm, J. B., Chen, B., & Rao, J. (2020). Viability of *Lactobacillus rhamnosus* GG microencapsulated in alginate/chitosan hydrogel particles during storage and simulated gastrointestinal digestion: Role of chitosan molecular weight. *Soft Matter*, *16*(7), 1877–1887.

Ragavan, M. L., & Das, N. (2018). Process optimization for microencapsulation of probiotic yeasts. *Frontiers in Biology*, *13*(3), 197–207.

Rajam, R., & Anandharamakrishnan, C. (2015). Microencapsulation of *Lactobacillus plantarum* (MTCC 5422) with fructooligosaccharide as wall material by spray drying. *LWT-Food Science and Technology*, *60*(2), 773–780.

Raman, M., Ambalam, P., & Doble, M. (2019). Probiotics, prebiotics, and fibers in nutritive and functional beverages. In *Nutrients in beverages* (pp. 315–367). United States: Academic Press.

Ranadheera, C. S., Evans, C. A., Baines, S. K., Balthazar, C. F., Cruz, A. G., Esmerino, E. A., et al. (2019). Probiotics in goat milk products: Delivery capacity and ability to improve sensory attributes. *Comprehensive Reviews in Food Science and Food Safety*, *18*(4), 867–882.

Reale, A., Di Renzo, T., & Coppola, R. (2019). Factors affecting viability of selected probiotics during cheese-making of pasta filata dairy products obtained by direct-to-vat inoculation system. *LWT*, *116*, 108476.

Ren, H., Yu, R., Qi, J., Zhang, L., Jin, Q., & Wang, D. (2019). Hollow multishelled heterostructured anatase/TiO_2 (B) with superior rate capability and cycling performance. *Advanced Materials*, *31*(10), 1805754.

Reyes, V., Chotiko, A., Chouljenko, A., & Sathivel, S. (2018). Viability of *Lactobacillus acidophilus* NRRL B-4495 encapsulated with high maize starch, maltodextrin, and gum arabic. *LWT*, *96*, 642–647.

Riaz, T., Iqbal, M. W., Saeed, M., Yasmin, I., Hassanin, H. A., Mahmood, S., et al. (2019). In vitro survival of *Bifidobacterium bifidum* microencapsulated in zein-coated alginate hydrogel microbeads. *Journal of Microencapsulation*, *36*(2), 192–203.

Roberts, D., Reyes, V., Bonilla, F., Dzandu, B., Liu, C., Chouljenko, A., et al. (2018). Viability of *Lactobacillus plantarum* NCIMB 8826 in fermented apple juice under simulated gastric and intestinal conditions. *LWT-Food Science and Technology*, *97*, 144–150.

Rodrigues, S., Silva, L. C., Mulet, A., Cárcel, J. A., & Fernandes, F. A. (2018). Development of dried probiotic apple cubes incorporated with *Lactobacillus casei* NRRL B-442. *Journal of Functional Foods*, *41*, 48–54.

Romero-Luna, H. E., Hernández-Sánchez, H., Ribas-Aparicio, R. M., Cauich-Sánchez, P. I., & Dávila-Ortiz, G. (2019). Evaluation of the probiotic potential of *Saccharomyces cerevisiae* strain (C41) isolated from tibicos by in vitro studies. *Probiotics and Antimicrobial Proteins*, *11*(3), 794–800.

Roobab, U., Batool, Z., Manzoor, M. F., Shabbir, M. A., Khan, M. R., & Aadil, R. M. (2020). Sources, formulations, advanced delivery and health benefits of probiotics. *Current Opinion in Food Science*, *32*, 17–28.

Ryan, J., Hutchings, S. C., Fang, Z., Bandara, N., Gamlath, S., Ajlouni, S., et al. (2020). Microbial, physico-chemical and sensory characteristics of mango juice-enriched probiotic dairy drinks. *International Journal of Dairy Technology*, *73*(1), 182–190.

Salami, M., Kouchaki, E., Asemi, Z., & Tamtaji, O. R. (2019). How probiotic bacteria influence the motor and mental behaviors as well as immunological and oxidative biomarkers in multiple sclerosis? A double blind clinical trial. *Journal of Functional Foods*, *52*, 8–13.

Sanap, D. S., Garje, M. A., & Godge, G. R. (2019). Probiotics, their health benefits and applications for development of human health: A review. *Journal of Drug Delivery and Therapeutics*, *9*(4-s), 631–640.

Sanders, M. E., Merenstein, D., Merrifield, C. A., & Hutkins, R. (2018). Probiotics for human use. *Nutrition Bulletin*, *43*(3), 212–225.

Sartor, R. B. (2004). Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: Antibiotics, probiotics, and prebiotics. *Gastroenterology*, *126*(6), 1620–1633.

Schepper, J. D., Irwin, R., Kang, J., Dagenais, K., Lemon, T., Shinouskis, A., et al. (2017). Probiotics in gut-bone signaling. In *Understanding the gut-bone signaling axis* (pp. 225–247). Cham: Springer.

Schoina, V., Terpou, A., Papadaki, A., Bosnea, L., Kopsahelis, N., & Kanellaki, M. (2020). Enhanced aromatic profile and functionality of cheese whey beverages by incorporation of probiotic cells immobilized on *Pistacia terebinthus* resin. *Foods*, *9*(1), 13.

Semyonov, D., Ramon, O., Kovacs, A., Friedlander, L., & Shimoni, E. (2012). Air-suspension fluidized-bed microencapsulation of probiotics. *Drying Technology*, *30*(16), 1918–1930.

Serrano-Niño, J. C., Cavazos-Garduño, A., Hernandez-Mendoza, A., Applegate, B., Ferruzzi, M. G., San Martin-González, M. F., et al. (2013). Assessment of probiotic strains ability to reduce the bioaccessibility of aflatoxin M1 in artificially contaminated milk using an in vitro digestive model. *Food Control*, *31*(1), 202–207.

Shahrampour, D., Khomeiri, M., Razavi, S. M. A., & Kashiri, M. (2020). Development and characterization of alginate/pectin edible films containing *Lactobacillus plantarum* KMC 45. *LWT*, *118*, 108758.

Shin, Y., Kang, C. H., Kim, W., & So, J. S. (2019). Heat adaptation improved cell viability of probiotic *Enterococcus faecium* HL7 upon various environmental stresses. *Probiotics and Antimicrobial Proteins*, *11*(2), 618–626.

Shit, S. C., & Shah, P. M. (2014). Edible polymers: Challenges and opportunities. *Journal of Polymer*, *2014*.

Shori, A. B. (2017). Microencapsulation improved probiotics survival during gastric transit. *HAYATI Journal of Biosciences*, *24*(1), 1–5.

Siezen, R. J., & Kleerebezem, M. (2011). The human gut microbiome: Are we our enterotypes? *Microbial Biotechnology*, *4*(5), 550–553.

Singh, P., Magalhães, S., Alves, L., Antunes, F., Miguel, M., Lindman, B., et al. (2019). Cellulose-based edible films for probiotic entrapment. *Food Hydrocolloid*, *88*, 68–74.

Šipailienė, A., & Petraitytė, S. (2018). Encapsulation of probiotics: Proper selection of the probiotic strain and the influence of encapsulation technology and materials on the viability of encapsulated microorganisms. *Probiotics and Antimicrobial Proteins*, *10*(1), 1.

Sivamaruthi, B. S., Kesika, P., Suganthy, N., & Chaiyasut, C. (2019). A review on role of microbiome in obesity and antiobesity properties of probiotic supplements. *BioMed Research International*, *2019*.

Soares, M. B., Martinez, R. C., Pereira, E. P., Balthazar, C. F., Cruz, A. G., Ranadheera, C. S., et al. (2019). The resistance of *Bacillus*, *Bifidobacterium*, and *Lactobacillus* strains with claimed probiotic properties in different food matrices exposed to simulated gastrointestinal tract conditions. *Food Research International*, *125*, 108542.

Sohail, A., Turner, M. S., Prabawati, E. K., Coombes, A. G., & Bhandari, B. (2012). Evaluation of *Lactobacillus rhamnosus* GG and *Lactobacillus acidophilus* NCFM encapsulated using a novel impinging aerosol method in fruit food products. *International Journal of Food Microbiology*, *157*(2), 162–166.

Song, H., Zhang, J., Qu, J., Liu, J., Yin, P., Zhang, G., et al. (2019). *Lactobacillus rhamnosus* GG microcapsules inhibit *Escherichia coli* biofilm formation in coculture. *Biotechnology Letters*, *41*(8-9), 1007–1014.

Soukoulis, C., Yonekura, L., Gan, H. H., Behboudi-Jobbehdar, S., Parmenter, C., & Fisk, I. (2014). Probiotic edible films as a new strategy for developing functional bakery products: The case of pan bread. *Food Hydrocolloid*, *39*, 231–242.

Speranza, B., Petruzzi, L., Bevilacqua, A., Gallo, M., Campaniello, D., Sinigaglia, M., et al. (2017). Encapsulation of active compounds in fruit and vegetable juice processing: Current state and perspectives. *Journal of Food Science*, *82*(6), 1291–1301.

Sugiharto, S., Yudiarti, T., Isroli, I., Widiastuti, E., Wahyuni, H. I., & Sartono, T. A. (2019). Effect of formic acid, *Saccharomyces cerevisiae* or their combination on the growth performance and serum indices of the Indonesian indigenous crossbred chickens. *Annals of Agricultural Sciences*, *64*(2), 206–210.

Suryabhan, P., Lohith, K., & Anu-Appaiah, K. A. (2019). Sucrose and sorbitol supplementation on maltodextrin encapsulation enhance the potential probiotic yeast survival by spray drying. *LWT*, *107*, 243–248.

Talwalkar, A., Kailasapathy, K., Peiris, P., & Arumugaswamy, R. (2001). Application of RBGR-A simple way for screening of oxygen tolerance in probiotic bacteria. *International Journal of Food Microbiology*, *71*(2-3), 245–248.

Terpou, A., Papadaki, A., Lappa, I. K., Kachrimanidou, V., Bosnea, L. A., & Kopsahelis, N. (2019). Probiotics in food systems: Significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. *Nutrients*, *11*(7), 1591.

Valdés-Varela, L., Gueimonde, M., & Ruas-Madiedo, P. (2018). Probiotics for prevention and treatment of *Clostridium difficile* infection. In *Updates on clostridium difficile in Europe* (pp. 161–176). Cham: Springer.

Vargas, L. A., Olson, D. W., & Aryana, K. J. (2015). Whey protein isolate improves acid and bile tolerances of *Streptococcus thermophilus* ST-M5 and *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12. *Journal of Dairy Science*, *98*(4), 2215–2221.

Vong, W. C., & Liu, S. Q. (2019). The effects of carbohydrase, probiotic *Lactobacillus paracasei* and yeast *Lindnera saturnus* on the composition of a novel okara (soybean residue) functional beverage. *LWT*, *100*, 196–204.

Wang, B., Yao, M., Lv, L., Ling, Z., & Li, L. (2017). The human microbiota in health and disease. *Engineering*, *3*(1), 71–82.

Wang, J., Korber, D. R., Low, N. H., & Nickerson, M. T. (2015). Encapsulation of *Bifidobacterium adolescentis* cells with legume proteins and survival under stimulated gastric conditions and during storage in commercial fruit juices. *Food Science and Biotechnology*, *24*(2), 383–391.

Wang, M., Yang, J., Li, M., Wang, Y., Wu, H., Xiong, L., et al. (2019). Enhanced viability of layer-by-layer encapsulated *Lactobacillus pentosus* using chitosan and sodium phytate. *Food Chemistry*, *285*, 260–265.

Wen, P., Zong, M. H., Linhardt, R. J., Feng, K., & Wu, H. (2017). Electrospinning: A novel nano-encapsulation approach for bioactive compounds. *Trends in Food Science and Technology*, *70*, 56–68.

Westfall, S., Lomis, N., & Prakash, S. (2018). A polyphenol-rich prebiotic in combination with a novel probiotic formulation alleviates markers of obesity and diabetes in *Drosophila*. *Journal of Functional Foods*, *48*, 374–386.

Xavier, J. R., Madhankumar, M. M., Natarajan, G., Ramana, K. V., & Semwal, A. D. (2019). Optimized production of poly γ-glutamic acid (γ-PGA) using *Bacillus licheniformis* and its application as cryoprotectant for probiotics. *Biotechnology and Applied Biochemistry*.

Xiao, J., Wang, T., Xu, Y., Gu, X., Li, D., Niu, K., et al. (2020a). Long-term probiotic intervention mitigates memory dysfunction through a novel H3K27me3-based mechanism in lead-exposed rats. *Translational Psychiatry*, *10*(1), 1–18.

Xiao, Y., Han, C., Yang, H., Liu, M., Meng, X., & Liu, B. (2020b). Layer (whey protein isolate)-by-layer (xanthan gum) microencapsulation enhances survivability of *L. bulgaricus* and *L. paracasei* under simulated gastrointestinal juice and thermal conditions. *International Journal of Biological Macromolecules*, *148*, 238–247.

Yao, M., Li, B., Ye, H., Huang, W., Luo, Q., Xiao, H., et al. (2018). Enhanced viability of probiotics (*Pediococcus pentosaceus* Li05) by encapsulation in microgels doped with inorganic nanoparticles. *Food Hydrocolloid*, *83*, 246–252.

Yasmin, I., Saeed, M., Pasha, I., & Zia, M. A. (2019). Development of whey protein concentrate-pectin-alginate based delivery system to improve survival of *B. longum* BL-05 in simulated gastrointestinal conditions. *Probiotics and Antimicrobial Proteins*, *11*(2), 413–426.

Ye, J., Ma, D., Qin, W., & Liu, Y. (2018). Physical and antibacterial properties of sodium alginate-Sodium carboxymethylcellulose films containing *Lactococcus lactis*. *Molecules*, *23*(10), 2645.

Yodsenee, K., Showpanish, K., Sonhom, N., Pilasombut, K., Prachom, N., Sathitkowitchai, W., et al. (2020). Encapsulation of *Pediococcus pentosaceus* RSU-Nh1 into pectin-sodium alginate and chitosan coating. *Technology*, *16*(1), 207–222.

Yucel Falco, C., Amadei, F., Dhayal, S. K., Cárdenas, M., Tanaka, M., & Risbo, J. (2019). Hybrid coating of alginate microbeads based on protein-biopolymer multilayers for encapsulation of probiotics. *Biotechnology Progress*, *35*(3), e2806.

Zaeim, D., Sarabi-Jamab, M., Ghorani, B., & Kadkhodaee, R. (2019). Double layer co-encapsulation of probiotics and prebiotics by electro-hydrodynamic atomization. *LWT*, *110*, 102–109.

Zaeim, D., Sarabi-Jamab, M., Ghorani, B., Kadkhodaee, R., & Tromp, R. H. (2017). Electrospray assisted fabrication of hydrogel microcapsules by single-and double-stage procedures for encapsulation of probiotics. *Food and Bioproducts Processing*, *102*, 250–259.

Zanjani, M. A. K., Ehsani, M. R., Ghiassi Tarzi, B., & Sharifan, A. (2018). Promoting *Lactobacillus casei* and *Bifidobacterium adolescentis* survival by microencapsulation with different starches and chitosan and poly L-lysine coatings in ice cream. *Journal of Food Processing and Preservation*, *42*(1), e13318.

Zeng, X., Fan, J., He, L., Duan, Z., & Xia, W. (2019). Technological properties and probiotic potential of yeasts isolated from traditional low-salt fermented Chinese fish Suan yu. *Journal of Food Biochemistry*, *43*(8), e12865.

Zhang, Q., Wu, Y., & Fei, X. (2016). Effect of probiotics on glucose metabolism in patients with type 2 diabetes mellitus: A meta-analysis of randomized controlled trials. *Medicina-Lithuania*, *52*(1), 28–34.

Zhang, T., Li, Q., Cheng, L., Buch, H., & Zhang, F. (2019). *Akkermansia muciniphila* is a promising probiotic. *Microbial Biotechnology*, *12*(6), 1109–1125.

Zhao, J., Ling, Y., Zhang, R., Ke, C., & Hong, G. (2018). Effects of dietary supplementation of probiotics on growth, immune responses, and gut microbiome of the abalone *Haliotis diversicolor*. *Aquaculture*, *493*, 289–295.

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Chapter 30

Industrial Requirements and Other Techno-functional Traits of Probiotics

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1 Introduction

Probiotics are live microorganisms that can be formulated into different types such as drugs, foods, and dietary supplements (Arvanitoyannis & Van Houwelingen-Koukaliaroglou, 2005). Probiotic is used to represent the beneficial bacteria for the animals, plants, and humans. *Bifidobacterium*, *Lactobacillus*, *Escherichia coli*, *Bacillus* species, and *Saccharomyces cerevisiae* are used to formulate the probiotics as food and feed (Table 30.1). Particularly, lactic acid bacteria (LAB) are used for fermenting the bacteria that can serve as food fermenting agent (Krockel, 2006; MacNeil, 2005). Generally, the probiotic strain exhibits some desirable characteristics such as low cost, maintaining its viability during the processing and storage facility.

TABLE 30.1 List of probiotic species.

List of probiotic species	Group of microbes	References
Lactobacillus acidophilus, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *Lactobacillus brevis*, *Lactobacillus johnsonii*, *Lactobacillus plantarum*, *Lactobacillus salivarius*, *Lactobacillus fermentum*, *Lactobacillus kefiri*	Lactic acid producing bacteria	Bayane et al. (2010), Bermudez-Brito, Plaza-Diaz, Munoz-Quezada, Gomez-Llorente, and Gil (2012), Chalas et al. (2016), Sharifi Yazdi, Davoodabadi, Khesht Zarin, Tajabadi Ebrahimi, and Soltan Dallal (2017), Taverniti, Scabiosi, Arioli, Mora, and Guglielmetti (2014)
Enterococcus faecalis, *Enterococcus faecium*, *Escherichia coli*, *Streptococcus thermophilus*, *Propionibacterium*	Nonlactic acid producing bacteria	
Bifidobacterium adolescentis, *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium lactis*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *B. bifidum*	*Bifidobacterium* species	
Saccharomyces boulardii	Nonpathogenic yeast	
Bacillus subtilis, *Saccharomyces cerevisiae*, *Candida pintolopesii*, *Aspergillus niger*, *Aspergillus oryzae*, *Bacillus licheniformis*, *Bacillus cereus var. toyoi*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus laterosporus*, *Bacillus pumilus*, *Bacillus racemilacticus*, *S. thermophilus*	Non-spore forming	
S. thermophilus, *Streptococcus salivarius*,	*Streptococcus* species	
S. boulardii	*Saccharomyces* species	

Advances in Probiotics. http://dx.doi.org/10.1016/B978-0-12-822909-5.00030-7
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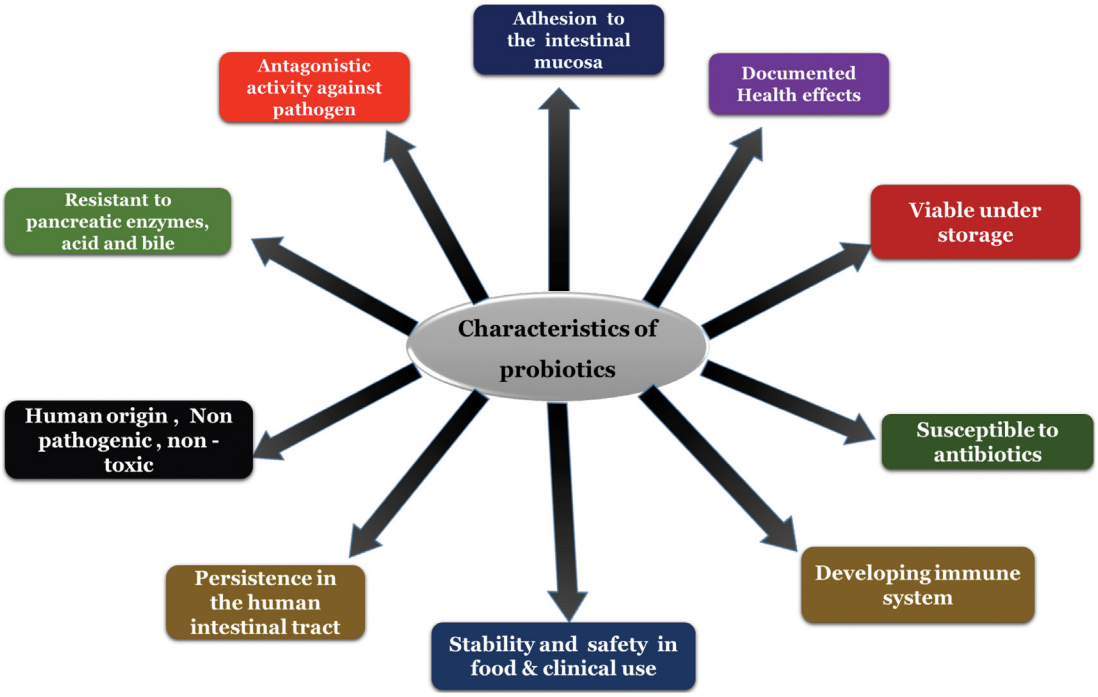


FIGURE 30.1 Characteristics of probiotics.

1.1 Characteristics of probiotics

Probiotics have the characteristics of surviving in the upper digestive tract, colonizing in the intestinal lumen and colon at the undefined time period. No studies have found that probiotics are toxic to human beings (Von Wright & Axelsson, 2012). The most probiotic strains are catalase-negative, Gram-positive, and rods with rounded ends, which occur as long chains, short (or) pairs. They are nonmotile, no spore formation, and are not salt tolerant (Von Wright & Axelsson, 2012). Conditions for growing probiotics are *T*—37°C and pH—6.5–7.0. *Lactobacillus acidophilus* is microaerophilic organism, and *Bifidobacterium* is anaerobic. But some species are aero-tolerant. Probiotic strains are either obligate heterofermentative (*Lactobacillus brevis*, *Lactobacillus reuteri*) or facultative heterofermentative (*Lactobacillus casei*, *Lactobacillus plantarum*) or obligate homofermentative (*L. acidophilus*, *Lactobacillus helveticus*) (Barrangou, Lahtinen, Ibrahim, & Ouwehand, 2012). It produces the beneficial compounds such as lactic acid, hydrogen peroxide, antimicrobials, and a variety of bacteriocins. In addition, probiotics have the ability to interact with the host microflora and be competitive with other microbial pathogens such as viral, bacterial, and fungal (Gorbach, 2002). Some probiotic strains can improve the quality of migrating motor complexes, reduce intestinal transit time, and increase mitosis rate in enterocytes (Banasaz, Norin, Holma, & Midtvedt, 2002) (Fig. 30.1).

2 Health benefits of probiotics

Probiotics provide health benefits to animal, human, and plants. However, the viability of microorganisms is important throughout processing and storage. In addition, health benefits will be based on the characteristics of probiotics.

Probiotics provide health benefits with a high nutritional value (Hotel & Cordoba, 2001). It has been reported by research groups and published in journals. Health benefits of probiotic strains are specific and also, there is no strain possessing all the health benefits, and not all the strains of the same species are effective against health conditions (Vasiljevic & Shah, 2008). Established probiotics for human health are *L. acidophilus*, *Bifidobacterium* sp., and *L. casei* whereas *S. cerevisiae*, some *E. coli* and *Bacillus* species are also exploited as probiotics (De Vrese & Schrezenmeir, 2008) (Table 30.2). Moreover, antimutagenicity, antigenotoxicity, and anticarcinogenicity are the most important properties of probiotics. Probiotics are used to assist naturally occurring microbes present in gut. In addition, some probiotics are used to prevent the diarrhea. Some reports are evidenced that probiotics help in lowering cholesterol. Probiotics have the ability to reduce the risk of cancer by inhibiting pro-carcinogens into carcinogens (Vasiljevic & Shah, 2008).

TABLE 30.2 Examples of probiotics and its beneficial effect.

S. no.	Microorganism	Beneficial effects	References
1.	*Lactobacillus GG*, *Lactobacillus rhamnosus*, *Lactobacillus fermentum*, *Bifidobacterium bifidum*, *Bifidobacterium lactis*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus salivarius*, and *Lactobacillus lactis*	• Immune modulation • Prevention of urogenital infections • Prevention of diarrhea of *Clostridium difficile* • Prevention of diarrhea of rotavirus	Doege et al. (2012), Yeşilova, Çalka, Akdeniz, and Berktaş (2012)
2.	*Bifidobacterium animalis* subsp.	• Protective effect against diarrhea • Reduce side effects of antibiotic treatment (antibiotic associated diarrhea) • Reduce the incidence of acute respiratory tract infections	Guerra et al. (2011), Sadeghzadeh, Rabieefar, Khoshnevisasl, Mousavinasab, and Eftekhari (2014)
3.	*L. casei*, *L. rhamnosus*, *Streptococcus thermophilus*, *Bifidobacterium breve*, *L. acidophilus*, *Bifidobacterium infantis*, *Lactobacillus delbrueckii* subsp. *bulgaricus*	• Immune modulation • Improvement of microbiota's function • Protective effect against diarrhea • Very effective in reducing colic in breastfed infants and children	Chau et al. (2015), Kianifar et al. (2014)
4.	*Saccharomyces boulardii*, *L. rhamnosus*, *L. rhamnosus GG*, *B. animalis* subsp. *lactis* with *Streptococcus. L. acidophilus*, *L. casei*	• Prevention of diarrhea • Decrease in the duration of acute gastroenteritis • Prevention of diarrhea of *C. difficile*	Guandalini (2011), Phavichitr, Puwdee, and Tantibhaedhyangkul (2013)
5.	*Bacillus clausii*, *Lactobacillus*, *Bifidobacterium*, and *Lactobacillus johnsonii*	• Anti-*Helicobacter pylori* effect	Hsieh et al. (2012), Vandenplas, Huys, and Daube (2015)
6.	*Lactobacillus reuteri*	• Antidiarrheal effect in children	
7.	*Oxalobacter formigenes*, *Lactobacillus*, and *Bifidobacterium* species	• Metabolize and degrade a toxic compound	Mogna, Pane, Nicola, and Raiteri (2014), Siener et al. (2013)
8.	*L. acidophilus*, *Lactobacillus gasseri*, *L. rhamnosus*	• Decrease the risk of type two diabetes mellitus and insulin resistance	Andreasen et al. (2010), da Silva, dos Santos, and Bressan (2013)
9.	*L. rhamnosus*, *L. reuteri*, *Propionibacterium freudenreichii*	Used to treat fungal pathogen	Haukioja (2010), Jørgensen, Kragelund, Jensen, Keller, and Twetman (2017)
10.	*B. animalis* subsp. *lactis*, *L. lactis* subsp. *lactis*	Modulation of brain activity and provide mental health	Liu, Cao, and Zhang (2015), Tillisch et al. (2013)

In addition, the microflora present in the gastrointestinal tracts of animals is very important in normal digestive processes and helps one to maintain the health of the animals. Probiotics can beneficially improve the intestinal microbial balance in host animal. Probiotics are used to enhance the milk production in dairy cows, feed efficiency in feedlot cattle, improve health and performance of young calves, and growth performance of chickens (Kalavathy, Abdullah, Jalaludin, & Ho, 2003). Moreover, probiotics attach the mucosal growth performance of chickens. It provides the nutrients and digestive enzymes to animals (Wang, 2007). Further, they can induce vitamin B synthesis and enhance the growth of nonpathogenic Gram-positive anaerobic bacteria by secreting inhibitory compounds and hydrogen peroxide that inhibit the harmful bacteria (Wang, 2007). Apart from this, probiotics enhance the uptake of organic materials and improve the immune response against pathogens (Balcazar, Rojas-Luna, & Cunningham, 2007). Finally, probiotics can inhibit toxic compounds produced by pathogens or stimulate the immune system.

Probiotics can serve as biocontrol agents to the plants in which probiotic bacteria and fungi are isolated and developed commercially to control the plant diseases. These kinds of probiotic strains induce immunity, antagonize various plant pathogens, and promote growth. Several studies reported the interaction between bacteria and fungi with the host plants (Compant, Clément, & Sessitsch, 2010; Sheng, Xia, Jiang, He, & Qian, 2008) (Fig. 30.2).

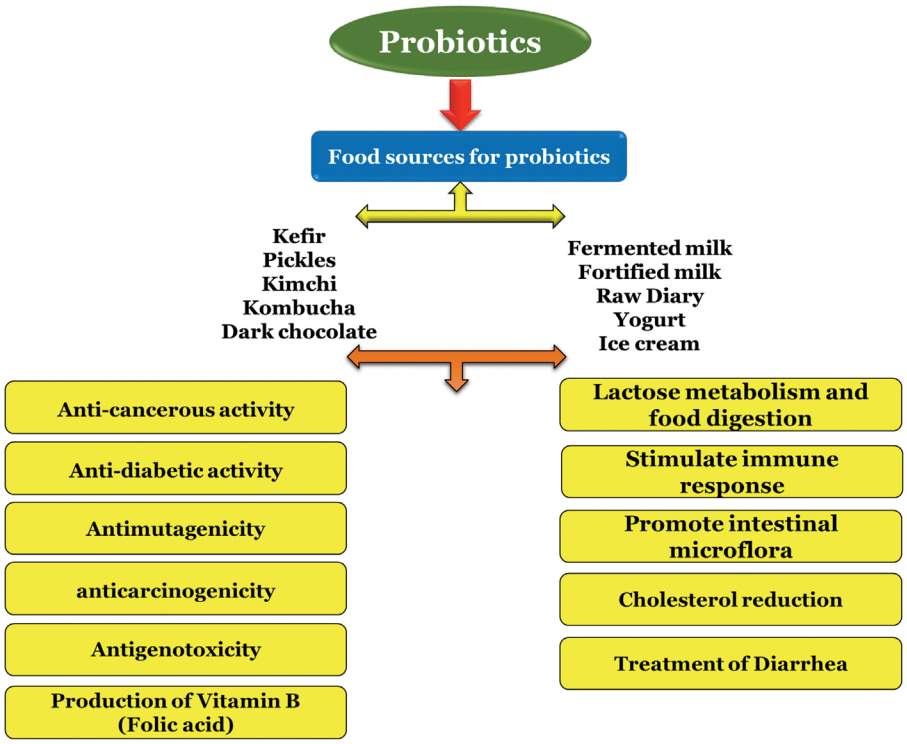


FIGURE 30.2 Health benefits of probiotics.

3 The techno-functional traits approaches of probiotics

In various pharmaceutical industries, LAB are frequently used as an important probiotic. They have been isolated frequently from various fermented food in the entire world (Barrangou, Lahtinen, Ibrahim, & Ouwehand, 2012). Based on the effect of probiotic nature, the LAB are used in food and dairy industry worldwide. Its very safe for consumption for human upon confirmation from the guidelines as well as once bacteria are detected of potential probiotic nature. LAB made from fermented food have been closed to some probiotic characteristic features such as increasing the intolerance of lactose level followed by digestion, reducing the level of cholesterol present in blood serum, suppressing cancerous cells, and increasing resistance to infections in the gastrointestinal and urogenital tracts (Deliza, Rosenthal, & Silva, 2003). In traditionally fermented food and beverages production, LAB acted as functional foods with high probiotic potentiality have been contributed significantly by the presence of LAB. The implementation of LAB is very useful in health system, because of their important characteristic features such as nontoxic, antipathogenic, functional, and technological attributes. It has stimulated research interests worldwide (Doege et al., 2012). It also provides the advantages in physiological nature to human, including retaining intestinal microbiota balance, inhibition of pathogens, boosting immunity, removal of carcinogen from intestinal tract, lactose tolerance, and biotransformation of nutrients availability to host.

In addition, the taste and aroma of fermented foods have played an important role and also have different biological factors particularly the microbial quality. LAB have extraordinary characters as probiotic in various pharmaceutical industries for fermented foods (Ahmed, 2003). They have been used with starter formulation, which is an important tool to preserve the exemplary properties of fermented food products. Therefore the techno-functional characteristics of some bacteria in fermented foods would be a matter of interest with tangible benefits. Interestingly, LAB have divulging cogent probiotic traits and have been used in various pharmaceutical industries (Aragon-Alegro, Alegro, Cardarelli, Chiu, & Saad, 2007). They improve the sensory properties of fermented foods through the process of reducing nitrates to nitrite and finally molecules of nitrous oxide. Additionally, they interfere in rancidity production by peroxide decomposition. Further, the proteolysis and lipolysis processes are used to change the flavor and aroma compounds in fermented foods.

The ability to determine the potential of carbohydrate fermentation includes fermentation capabilities of glucose, lactose, dextrose, maltose, fructose, and sucrose. Sometimes the production of exopolysaccharides by LAB has been used for various food and dairy industries (Bermúdez-Humarán et al., 2015). In addition, the lipolytic activity of amylase producing LAB enhances the probiotic nature in all pharmaceutical industries. The production of high-ratio probiotic natured bacteria

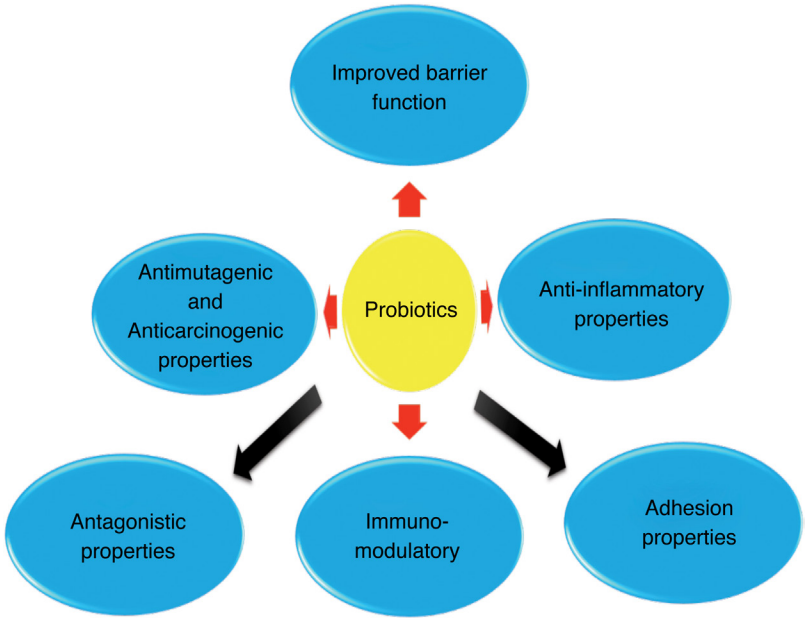


FIGURE 30.3 Functional aspects of probiotics.

has been stimulated by autolysis in all industries for higher yield. After careful consideration, nitrate and catalysis–reduced LAB have been frequently used in pharmaceutical industries for improved fermented food (Chichlowski, Croom, McBride, Havenstein, & Koci, 2007). Some functional aspects of probiotics are depicted in Fig. 30.3.

3.1 Functional aspects of probiotics

Some of the functional aspects of probiotics are discussed in more detail later. Adhesion, antagonism, immunomodulatory, antimutagenic, and anticarcinogenic properties are the important properties of probiotics.

3.2 Adhesion properties

Probiotics can reduce the pathogenic microorganisms by binding with the surface of the host cells at the gastrointestinal tract by adhering to the space (Ohashi & Ushida, 2009). Pathogenic microorganisms require nutrients to multiply by alleviating infections. Generally, gastrointestinal tract is a suitable environment for microbial colonization where probiotics compete with the pathogen for nutrients and colonize their growth at the site such as goblet cells, the intestinal villi, and the colonic crypts (Chichlowski et al., 2007). During competition for nutrients, probiotics secrete secondary metabolites that decrease the pH of the gastrointestinal tract that is not suitable for pathogenic growth. Adhesion and colonization of probiotic strains to the internal surface of gastrointernal tract are considered as important properties of probiotics. Adherent strains of probiotic bacteria continue to persist longer time in the intestinal tract via electrostatic interactions or specific surface proteins. Probiotic LAB adhere more to the epithelial cells than pathogens (Lee, Puong, Ouwehand, & Salminen, 2003). For example, *lactobacilli* and *bifidobacteria* attach with glycolipids on the surface of the host cells to prevent the attachment of certain pathogens (Wohlgemuth, Loh, & Blaut, 2010). It maintains its contact with gut-associated lymphoid tissue, influencing local and systemic immune effect. Thus adherent probiotics can induce immune effect and mucosal barrier.

3.3 Antagonistic properties

In order to compete with the pathogenic microorganisms, probiotics release antimicrobial compounds. Antimicrobial compounds such as acetic and lactic acids prevent diseases by pathogen metabolism and toxins production. The antipathogenic activity of probiotics is multifactorial (Servin, 2004). Additionally, probiotics can secrete antibacterial compounds such as H_2O_2 and bacteriocins (Fayol-Messaoudi, Berger, Coconnier-Polter, Lievin-Le Moal, & Servin, 2005). They may function as colonizing peptides by introducing probiotics into the intestinal epithelium. This competitive advantage allows for an

increase in the density of probiotic bacteria on the surface of the host intestines (Dobson, Cotter, Ross, & Hill, 2012). They can also act as killing peptides, by directly affecting pathogens. The previous reports have shown the antimicrobial activity of the bacteriocins such as lacticin, pediocin, and leucocin produced by *Helicobacter pylori* (Kim et al., 2003). Similarly, *L. acidophilus* reportedly produces metabolites such as acidophilin, lactocidin, and acidolin whereas *bifidobacteria* secrete bacteriocins that are able to significantly inhibit the growth of *H. pylori*. Therefore it is identified that intestinal cells of the host produce defensins that can prevent the pathogen function by aiding the protection of the intestinal barrier (Risoen, Rønning, Hegna, & Kolstø, 2004).

3.4 Immunomodulatory properties

Probiotics can remove pathogen by stimulating the host immunity to prevent bacterial growth causing intestinal diseases. They control the immune system of the host against harmful pathogens by the activating lymphocytes and producing antibodies (Ng, Hart, Kamm, Stagg, & Knight, 2009). Therefore it adheres to gut-associated lymphoid tissues by stimulating the immune cells such as macrophages, T cells, and B cells by inducing innate and adaptive immune response (Viaşu-Bolocan, Popescu, & Bica, 2013). Probiotic strains such as *Lactobacillus rhamnosus* and *L. plantarum* enhance the intestinal mucins that interrupt the pathogen to disturb the pathogen translocation. Probiotics recognize the immune system by inducing the Toll-like receptors. This induces various intracellular transduction cascades and enhances the cytokines. As a result of which antibodies are produced in the intestinal lumen, especially immunoglobulin A (IgA) that describes the first line defense against the infection and can prevent the pathogenic bacteria to the intestinal epithelia by causing agglutination. The previous study indicated that *L. casei* enhances the concentration of IgA in infants inducing diarrhea. In addition, the study reported that administrating *L. rhamnosus* enhances the humoral responses by stimulating the level of circulating IgG, IgA, and IgM in children with acute gastroenteritis (Ng et al., 2009).

3.5 Improved barrier function

The entry of pathogens needs to be prevented by maintaining integrity of intestinal barrier function, thereby causing local and systemic infection. Previous reports mentioned that the consumption of probiotics can maintain mucosal integrity, prevent chronic infection thereby protect the host. Probiotics reduce the paracellular permeability, prevent chronic inflammation, and protect the host by inducing innate immune response. Probiotics are used to repair the intestinal barrier caused by gut pathogens. It can induce mucous, chloride, and water secretion, and the binding together of submucosa cells by tight junctional proteins (Wohlgemuth et al., 2010). Goblet cells secrete mucin that is localized to the cell membrane. In the human intestinal lines, *Lactobacillus* species secrete mucin expression thus block cellular adhesion and invasion by pathogenic *E. coli* (Mack, Ahrné, Hyde, Wei, & Hollingsworth, 2003). The previous study reported that *L. acidophilus* increases the occludin expression (component of tight junction) in the gut mucosa of animals with cecal ligation.

3.6 Anti-inflammatory properties

Chronic inflammatory disorders known as inflammatory bowel diseases are mainly characterized by the inflammation of the digestive tract. The treatment of inflammatory bowel diseases is severely affected by side effects in patients. The previous study reported that probiotics counteract the inflammatory process by inducing Elafin (Bermúdez-Humarán et al., 2015) that is a protease inhibitor expressed in the intestinal epithelium involved in the reduction of inflammation. Bermudez-Humaran et al. reported that *Lactobacillus lactis* expressed elafin in mice suffering from colitis. Moreover, *L. rhamnosus* GG was found to induce IgA immune response of Crohn's disease patients (Malin, Suomalainen, Saxelin, & Isolauri, 1996), which improved the response of circulating antibody secreting cell response in children suffered with rotavirus diarrhea (Kaila et al., 1992).

3.7 Antimutagenic and anticarcinogenic properties

Probiotics play an important role in antimutagenic and anticarcinogenic activities such as production of antimutagenic compounds, modulation of procarcinogenic enzymes in the gut, binding and degradation of carcinogens, and suppression of tumors by an immune response (Hirayama & Rafter, 1999). These properties are distributed among bacterial species representing both Gram-positive and Gram-negative species. Further, nonviable cells are able to bind lactic acid bacterial strains (*Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Enterococcus*, and *Pediococcus* species) isolated from fermented dairy and nondairy products, which exhibit antimutagenic and anticarcinogenic activities. Moreover, nonviable bacterial cells

bind mutagens and carcinogens. In addition, probiotic strains are associated with the reduction of fecal enzymes involved in mutagen or carcinogen activation as fecal enzymes, such as beta-glucuronidase and nitroreductase, are capable of converting procarcinogens to carcinogens in the colon.

4 Industry-based probiotics application in various fields

4.1 Food applications of probiotics

At present situation, functional foods have developed the research interest due to its health benefits. Moreover, industries have focused on various applications of food products especially, in probiotics. Applications of probiotics in food products are summarized.

4.2 Dairy-based probiotic foods

Milk and milk products are carriers of probiotic strains that can be stored at refrigerated temperatures. Probiotics dairy products are sour and fresh milk, yogurt, and cheese. Dairy products provide a suitable environment by supporting their growth and viability to supply the probiotic bacteria to human (Phillips, Kailasapathy, & Tran, 2006; Ross, Fitzgerald, Collins, & Stanton, 2002). Many factors are required to address probiotics in dairy products such as viability of probiotics, physical and chemical properties of final products, and the regulations and labeling issues (Hotel & Cordoba, 2001).

4.3 Fresh milk and fermented milks

Functional dairy beverages are divided into two types: fortified dairy beverages such as fibers, prebiotics, probiotics, peptides, polyphenols, sterol, stanols, vitamins, minerals, fish oil, and whey-based beverages (Özer & Kirmaci, 2010). Among the probiotic bacteria, *L. rhamnosus* is suitable for industrial applications. *L. casei*, *L. rhamnosus*, *L. acidophilus*, and *L. plantarum* are the mostly applied probiotics. Several factors such as acidity, redox potential, pH, starter microbes, and additives affect the viability of probiotic strains during the manufacture and storage of dairy products (Phillips et al., 2006; Saarela, 2009; Shah, 2000). Probiotics beverages are available in the market such as Yakult, Actimel, ProViva, sweet acidophilus milk, and acidophilus milk (Özer & Kirmaci, 2010). Dairy drinks are first commercial products that are consumed at larger quantities than other probiotics. Due to their low proteolytic activity, *Lactobacillus* and *Bifidobacterium* strains will not grow in milk (Saarela, 2009). Additionally, viability of probiotic bacteria in fermented milk is important Therefore selecting the probiotic strains and production conditions should be considered as very important factors (Martinez-Villaluenga, Frias, Gomez, & Vidal-Valverde, 2006; Saarela, Virkajarvi, Alakomi, Sigvart-Mattila, & Matto, 2006).

4.3.1 Yogurt

Yogurt is one of the most popular probiotic products using the cultures of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* bacteria. Numerous factors such as dissolved oxygen, pH, probiotic bacteria, concentration of metabolites, buffering capacity of the media, temperature, growth promoters, inhibitors, and concentration of solutes may affect the survival of *Lactobacillus* and *Bifidobacterium* spp. in yogurt (Shah, 2000). In addition, decrease in the pH of the medium and organic acids accumulation affects the loss of viability of probiotic organism (Donkor, Henriksson, Vasiljevic, & Shah, 2006). Some characteristics of yogurt include flavor, acidity, appearance, and texture (Aryana & McGrew, 2007). However, encapsulation such as chitosan-coated alginate, alginate-pectin, plain alginate beads, alginate-starch, in whey protein-based matrix would enhance the viability and stability of probiotics in yogurt (Oliveira et al., 2009; Paseephol & Sherkat, 2009; Sandoval-Castilla, Lobato-Calleros, García-Galindo, Alvarez-Ramírez, & Vernon-Carter, 2010).

4.4 Other dairy-based products

Other dairy products such as frozen fermented dairy desserts, chocolate, ice cream, quark, and sour cream are the good medium for probiotics. Chocolate mousse is a good vehicle for *Lactobacillus paracasei* (Aragon-Alegro et al., 2007). Similarly, quark was examined with two probiotic cultures to develop its nutritional value and ensures with a high nutritious value such as lactose, protein, phosphorus, and fat partially in skimmed milk (Duric, Ilicic, Milanovic, Caric, & Tekic, 2007). Sour cream was investigated as a probiotic vehicle and the results showed that using sour cream as a probiotic carrier is proved feasible (Wilson, Seo, Shahbazi, & Ibrahim, 2004). Ice creams are one of the probiotic carriers with high potential

for use as probiotic vehicles. Cruz and others have reviewed the technological parameters involved in the production of probiotic ice creams (Cruz, Antunes, Sousa, Faria, & Saad, 2009). Several factors are needed to be controlled such as inoculums concentration, processing procedures, appropriate processing stage for the cultures to be added, appropriate selection of cultures, transport and storage temperatures. They concluded that products hold good viability for probiotics during the product storage period.

4.5 Fruit-based probiotic products

Nowadays, interest has been developed using fruit juice-based probiotic products. The fruits are rich in nutrients such as vitamins, antioxidants, minerals, and dietary fibers (Luckow & Delahunty, 2004). The fruit juice contains beneficial nutrients that can be ideal medium for probiotics. Selection of appropriate strains to probiotics provides healthy fruit juice. Various odor and aroma are reported when *L. plantarum* is added with orange juices. The flavors arisen by probiotics dissatisfy the consumer, which may be masked by 10% of fruit juices, mainly pineapple or mango juices.

Moreover, *Bifidobacterium longum* and *L. plantarum* are the suitable medium for the production of noni juice fermentation (Wang, Ng, Su, Tzeng, & Shyu, 2009). *L. plantarum*, *Lactobacillu delbruekii*, *L. paracasei*, and *L. acidophilus* are a suitable fermenting medium for pomegranate juice (Mousavi, Mousavi, Razavi, Emam-Djomeh, & Kiani, 2011). *L. casei* is a suitable medium for fermenting cashew apple juice during 42 days of refrigerated storage (Pereiraa, Maciela, & Rodriguesa, 2011).

4.6 Cereal-based probiotic products

Cereals are the important substrates for the growth of probiotic strains. Cereal-based probiotic products have health-benefiting microbes and potentially prebiotic fibers. Due to the complexity of nondigestible components present in cereals, certain factors are required to promote the growth of probiotics in cereals (Kedia, Wang, Patel, & Pandiella, 2007). So that combination of cereals and health-promoting bacteria is a challenging issue.

Previous reports reported that the strains such as *L. plantarum*, *Candida lambica*, and *Candida rugosa* isolated from a traditional Bulgarian cereal-based fermented beverage possess probiotic properties (Gotcheva et al., 2002). Similarly, Rozada-Sanchez and others have reported the probiotic growth using the strains of *Bifidobacterium* sp. and malt hydrolysate with the aim of manufacturing probiotic beverage (Rozada-Sánchez, Sattur, Thomas, & Pandiella, 2008).

4.7 Meat-based probiotic foods

Meat starter culture is the preparation of living or resting microorganisms that promote the desired metabolic activity. In which, LAB are commonly used as starter culture in meat producing (Hammes & Hertel, 1998). Commonly used meat starter cultures are *L. casei*, *Lactobacillus curvatus*, *Lactobacillus pentosus*, *L. plantarum*, *Lactobacillus sakei*, *Pediococcus acidilactici*, and *Pediococcus pentosaceus* (Työppönen, Petaja, & Mattila-Sandholm, 2003). Since the meat content of the sugars is very low, 0.4%–0.7% glucose and 0.5%–1.0% lactose are added to the sausage matrix. Dry sausages are used with LAB to confirm the quality and safety of the end products. Dry sausages are meat products without heating. Dry sausages are used as suitable carriers for meat-based probiotic products. Dry sausages are made from a mixture of beef and pork fat, frozen pork by adding sugar, salt, nitrite, nitrate, and ascorbates. During fermentation, the growth of microbes uses the oxygen material initially and decreases the pH to above 5.0 which serves as a problem for several Gram-negative bacterial species (Leistner, 2000). In addition, probiotic strains with antimicrobial effects on food are more successful than other commercially used bacteria. Finally, it is represented that dry sausages are considered as suitable carriers to probiotic strains.

5 Agricultural applications of probiotics

Applications of probiotics are extended from human applications to diversity of agricultural application. Plant probiotic products are used as biofertilizers, biopesticides, and phytostimulators that depend on the mode of action and effects (Berg, 2009). Advantages of plant probiotics over chemical pesticides and fertilizers are safe, reduced environmental damage, targeted activity, and decompose more quickly than conventional chemical pesticides (Berg, 2009). Growth of the plant can be enhanced between beneficial microbes and the host plant includes *Azospirillum*, *Serratia*, *Pseudomonas*, *Streptomyces* (De Vleesschauwer & Höfte, 2003; Preston, 2004; Zhao et al., 2003).

Various mechanisms play the role in probiotics–plant interaction. When probiotic strains enter into the plant, they recognize the host plant, adhere, invade, colonize, and grow. Furthermore, colonized probiotic bacteria can influence the

hormonal balance that induces various metabolic reactions (Berg, 2009). Along with these mechanisms, probiotic strains can supply macronutrients and micronutrients. They release various amino acids, organic acids, and carbohydrates in the rhizosphere by catalyzing root exudates (Compant et al., 2010). In addition, bacteria may involve in plant nutrition composition by releasing phosphorous from organic compounds and thereby induce the plant growth (Unno, Okubo, Wasaki, Shinano, & Osaki, 2005). Moreover, probiotics can decrease the activity of pathogenic microorganism through antagonistic activity termed "induced systemic resistance" (Van Loon, 2007). Generally, three groups of compositing bacteria such as *Bacillus* bacteria groups, LAB groups, and *Actinomycetes* groups are used either individually or combined groups. These bacterial species can secure the plant from hazards. In addition, these are effective in controlling fungi such as pythium, powdery mildew, downy mildew, and *plasmodiophora brassicae*.

6 Livestock applications of probiotics

Probiotic has provided a possible natural alternative to antibiotics in poultry production to produce foods of reliable quality and safety (Langhout, 2000). Animal probiotics are live microbial feed supplements that enhance the intestinal microbial balance in host animal (Ibrahim et al., 2010). They are applied as animal feed to develop the animal health and food safety with respect to poultry, ruminant, pig, and aquaculture. The microflorae in the gastrointestinal tract of poultry play an important role in digestive processes and in maintaining animal's health. Some probiotics as feed additives can affect the microbial population and health-promoting effect. Due to the unwanted side effect of antibiotics, an increasing interest has to be developed in poultry production. Similarly, probiotics as feed additives can affect microbial population and health-promoting effect. Application of probiotic to chicken feed increases the quality of the egg, that is, weight shell thickness, albumen weight, and specific gravity (Hashemipour, Khaksar, & Kermanshahi, 2011).

When probiotics are applied to the farm animals, pathogen load decreases. Feeding probiotic LAB and yeast to calve was found to promote the growth and suppress diarrhea in *Holstein calves* (Kawakami, Yamada, Nakanishi, & Cai, 2010). Gaggia reported that the applying probiotics and prebiotics in animal feeding will be safe to food production (Gaggìa, Mattarelli, & Biavati, 2010). Probiotics are used to decrease pathogen load and ameliorate gastrointestinal disease symptoms in pigs. Recent studies were supported that probiotics improve the number of beneficial bacteria, decrease pathogen load, and induce the immune cell response toward pathogens when compared to control. The application of probiotics in aquaculture depends on the producing antimicrobial metabolites and their ability to attach with intestinal mucus and reduce the pathogen load (*Aeromonas hydrophila* and *Vibrio alginolyticus* are commonly found pathogens in fish) (Vine et al., 2004). Feeding probiotic strain to shrimp was shown to reduce the disease caused by *Vibrio parahaemolyticus* (Balcazar et al., 2007).

7 Probiotics application challenges

Many challenges need to be faced during the package of probiotic food products such as probiotic strain selection, inoculation, their growth and their survival during processing, viability and functionality during storage, and effects on sensory properties. Some of the issues have been described in the following.

Challenges regarding the stability of the probiotic products during the probiotic production remain unsolved. Probiotic strains require suitable environmental conditions to grow such as environmental conditions (pH, suitable temperature, oxygen content, and water activity) and suitable media. During product manufacturing and storage, viability of the bacterial strains, probiotic stability and properties should be considered. In addition, probiotic strains should remain viable when they reach gastrointestinal tract. Therefore suitable culture medium and protectants are required to improve the efficiency of the probiotic product. Generally, most probiotic strains are anaerobes, so oxygen permeation should be hindered (Shah, Ding, Fallourd, & Leyer, 2010). Currently, fermented milk and yogurts are good vehicles for probiotic strains in market. Therefore producing the probiotic product is very critical to reproduce (e Silva & Freitas, 2014).

7.1 Viability and survival

Probiotics provide many health benefits. During refrigeration and storage of products, claimed health benefits are not achieved due to low number of viable cells. In addition, during the fermentation of probiotic products, pH is reduced, which spoils the probiotic products. Therefore probiotic bacteria should depend on dosage, viability, and particularly, it should survive in the gut and shelf life of the product (Kailasapathy & Chin, 2000; Shah, 2007). In order to avoid this, additional ingredients will be supplemented to support the viability throughout the entire processing. Reports have shown that compounds such as fructooligosaccharides, inulin, and oligosaccharides are strain specific, which have good impact on the viability of the probiotics.

The studies reported that fermented milk products were developed in the presence of raffinose using *Bifidobacteria* and *L. acidophilus*, thereby viability was retained (Martinez-Villaluenga et al., 2006). Moreover, probiotic products are added with pectin, mannitol, and maltodextrin, which are considered as a suitable medium for probiotic bacteria (Yeo & Liong, 2010). Inulin and fructooligosaccharides enhance the growth and viability of *L. acidophilus.* But it will not significantly increase the growth and viability of *Bifidobacterium* and *L. casei* (Rodrigues et al., 2011). During formulation, several factors should be considered such as antimicrobials, nutrients, oxygen permeability, water activity, temperature, and pH. The most important factor affecting probiotic viability is pH. The pH values of beverages are 4.4 ensuring the stability of the microbes. In addition, the packaging material should be an oxygen barrier to enhance the growth of anaerobic probiotic bacteria (*bifidobacteria*) (Saarela, Mogensen, Fonden, Mättö, & Mattila-Sandholm, 2000). Moreover, transportation, storage, and temperature are very important for survival of probiotic bacteria that can affect the shelf life of probiotic bacteria (Saarela et al., 2000). Spore-forming probiotic bacteria, for example, GanedenBC30, are not affected with viability and stability, which can be considered as a suitable choice for product development. It is being protected from genetic materials of cell, heat, and pressure during manufacturing processes. GanedenBC30 has the ability to withstand manufacturing processes and survive even at high pressure like extrusion and roll-forming—at high temperature processes, it includes baking, boiling; and at low temperature processes, it includes freezing, refrigeration.

7.2 Sensory acceptance

Probiotic foods must follow the sensory test as conventional foods. It is important to develop the sensory tests for probiotic foods that can be accompanied by specific sensory analyses. Sensory testing includes the characteristics of all changes with respect to time. Recent studies have reported the performance of probiotic products as compared to conventional products: milk fermented with *Bifidobacterium animalis* and *L. acidophilus* La-5, chocolate mousse with added inulin and *L. paracasei* (Aragon-Alegro et al., 2007), functional yogurt supplemented with *L. reuteri* RC-14 and *L. rhamnosus* GR-1 (Hekmat & Reid, 2006), curdled milk with inulin, and *L. acidophilus* (Hekmat & Reid, 2006).

Sensory methodologies are used in developing the probiotic foods. In many cases, the developed products are required to match similar commercial products. Metabolic products of the probiotic culture will produce negative odor and taste of the food. Acetic acid secreted by *Bifidobacterium* can produce vinegary flavor that influences the performance of the sensory assessments.

In addition, masking is one of the techniques used to reduce the flavors in food. New substances are added to the medium that mask the unwanted flavors arisen by probiotics. For example, tropical fruit juices, mainly, pineapple, mango, or passion fruit, which are used to mask probiotic flavors (Luckow, Sheehan, Fitzgerald, & Delahunty, 2006). Therefore repeated exposure and increased familiarity to sensory off-flavors involve consumer willingness in a positive way to consume probiotic juices. Nonsensory techniques are useful to enhance the sensory quality of products providing health benefits related with probiotic cultures. Health related to probiotic information has shown a vital tool in the consumer acceptance of a variety of probiotic food products. Ultimately, the microencapsulation of probiotics is used to prevent the flavor of cultures (Deliza et al., 2003; Rokka & Rantamäki, 2010).

8 The future of probiotics

Up to now, we have discussed about dairy-based products and nondairy beverages where the probiotics will make their impact. The future of probiotics includes the field of biotechnology, nanotechnology, bioinformatics, and genetic engineering, in which genome sequencing project is launched for probiotic microorganisms due to recently developed sequencing and bioinformatic technologies. This can be utilized to study the gene expression profile of the probiotic strain. Transcription profiling is used to study the gene responsible for probiotic survival (Klaenhammer, Barrangou, Buck, Azcarate-Peril, & Altermann, 2005). Technological properties of probiotic strains such as *Lactobacillus* and *Bifidobacterium* species can be tailored due to the genetic engineering development. However, safety issues are very important and seriously considered for each modified strain (Ahmed, 2003).

Nanotechnology in probiotics is an interesting and emerging area which is applied to the agriculture, food sector, drug delivery, and pharmaceuticals. The nanostructured food ingredients are useful to improve texture, consistency, and taste. Previous reports showed about safety, risk problem of nanomaterial, regulation in nanotechnology, routes for nanoparticles entering the body, and existing regulations in nanotechnology (Sozer & Kokini, 2009). Nanoencapsulation is the technology to pack the food ingredients by using the techniques such as nanoemulsions and nanocomposites that extend the shelf life of the product and deliver the probiotic bacteria to gastrointestinal tract where they bind with specific receptor (Sinha, Vinay, & Bhinge, 2008) and improve the viability of probiotic bacteria in acidic food products. The microencapsulation

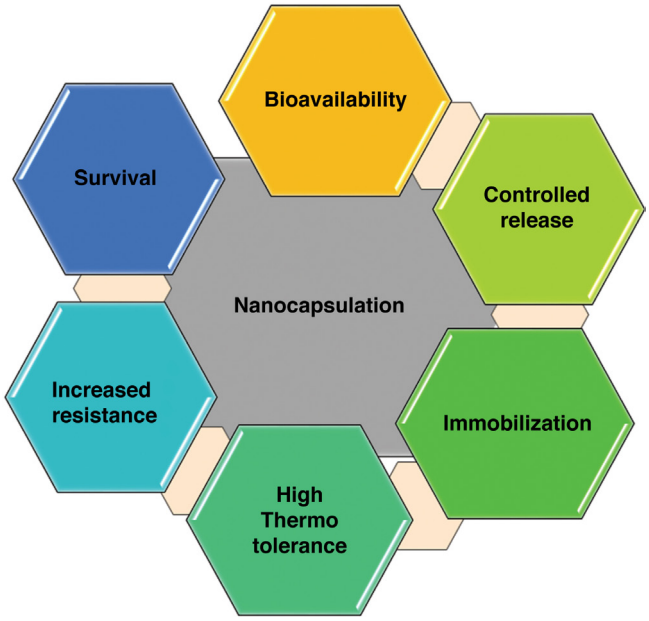


FIGURE 30.4 Advantages of nanocapsulation in probiotics.

involves in protecting the probiotic bacteria from outer environment by coating. Microencapsulation technologies are required for providing necessary protection to the probiotics, which will be useful to the pharmaceutical industry. Further, it can act as a supplement for the food ingredients. Microencapsulation with alginate is applied to many different probiotic strains at the pH of 2.0, *T*—65°C and high bile salt concentrations. Similarly, gelatin or vegetable gum is encapsulated with acid-sensitive *Bifidobacterium* and *Lactobacillus* (Chandramouli, Kailasapathy, Peiris, & Jones, 2004; Chen, Chen, & Lin, 2006) (Fig. 30.4).

9 Regulations and guidelines for probiotics

Regulatory requirements vary greatly depending upon the use of probiotics. If a probiotic product is considered to use as a drug, then it must follow the regulatory rules as a drug. Food and drug administration must be authorized to the newly investigated drug and its application. The probiotic drug should be proven safe and effective before marketing (Barrangou, Lahtinen, Ibrahim, & Ouwehand, 2012). In the United States, probiotics are supplied as food. Premarket notifications are required to the product. In addition to nutrient content claims, the law represents that manufacturers for probiotics should make functional food or healthy claims to their products.

9.1 Safety aspects and harmful side effects of probiotics

Probiotics causes four types of side effects—excessive immune stimulation, deleterious metabolic activities, gene transfer, and systemic infections. When doses of intake are very high, it causes infection to humans of all the age groups, including immuno-compromised individuals. Three approaches are used to assess the safety of the probiotic strains—(1) pharmacokinetics of the strain, (2) intrinsic properties of the strain, and (3) interaction between the strain and the host (Tan et al., 2017). It causes rare symptoms like bloating, flatulence, gurgling, stomach aches, and abnormal bowel movements. Available data indicate that not those much harmful effects have been observed in controlled clinical studies with *lactobacilli* and *bifidobacteria* (Doron & Snydman, 2015). The probiotics are metabolically active in the gastrointestinal tracts which are associated with the gastrointestinal mucosa and gastrointestinal microflora. Therefore probiotics should have some important properties such as (1) ability to translocate gastrointestinal tract—translocation are affected by various elements such as intestinal mucosal damage, immunodeficiency, abnormal bacterial flora, gut prematurity, and abnormal bacterial flora; (2) immunologic effects and metabolic activity of probiotics—sometimes, it causes deleterious effects by stimulating higher immune response; and (3) product quality—if there is no proper label of probiotics and appearance of contaminants, it causes danger to customer (Suresh, Krishnappa, & Bhardwaj, 2013).

10 Conclusion

Probiotic consumption leads to a healthy life. Probiotic uses and their applications in food and health have tremendously increased for the last two decades. Probiotics provide so many health benefits to the animals, plants, and humans. Probiotics face many health challenges. Moreover, apart from viability and sensory acceptance, some factors such as selection, inoculation of starter cultures, and processing must be considered as important for probiotics production. In addition, probiotic industry also faces new challenges during storage and transportation. In order to face these challenges, new studies are required for testing the ingredients, handling different media that have not yet been utilized, and further, lactose intolerant consumers are in demand with probiotic products.

Probiotics are widely used to solve and cure particular diseases. In the future, in vitro and in vivo experiments should be conducted to identify probiotics and to select the most suitable ones for the prevention (or) treatment of diseases. Lastly, the studies suggest that to proceed further, research needs confirmation about its effect in human health with advanced techniques and well-designed clinical trials.

References

Ahmed, F. E. (2003). Genetically modified probiotics in foods. *Trends in Biotechnology*, *21*(11), 491–497.

Andreasen, A. S., Larsen, N., Pedersen-Skovsgaard, T., Berg, R. M., Møller, K., Svendsen, K. D., et al. (2010). Effects of *Lactobacillus acidophilus* NCFM on insulin sensitivity and the systemic inflammatory response in human subjects. *British Journal of Nutrition*, *104*(12), 1831–1838.

Aragon-Alegro, L. C., Alegro, J. H. A., Cardarelli, H. R., Chiu, M. C., & Saad, S. M. I. (2007). Potentially probiotic and synbiotic chocolate mousse. *LWT—Food Science and Technology*, *40*(4), 669–675.

Arvanitoyannis, I. S., & Van Houwelingen-Koukaliaroglou, M. (2005). Functional foods: A survey of health claims, pros and cons, and current legislation. *Critical Reviews in Food Science and Nutrition*, *45*(5), 385–404.

Aryana, K. J., & McGrew, P. (2007). Quality attributes of yogurt with *Lactobacillus casei* and various prebiotics. *LWT—Food Science and Technology*, *40*(10), 1808–1814.

Balcazar, J. L., Rojas-Luna, T., & Cunningham, D. P. (2007). Effect of the addition of four potential probiotic strains on the survival of pacific white shrimp (*Litopenaeus vannamei*) following immersion challenge with *Vibrio parahaemolyticus*. *Journal of Invertebrate Pathology*, *96*(2), 147–150.

Banasaz, M., Norin, E., Holma, R., & Midtvedt, T. (2002). Increased enterocyte production in gnotobiotic rats mono-associated with *Lactobacillus rhamnosus* GG. *Applied and Environmental Microbiology*, *68*(6), 3031–3034.

Barrangou, R., Lahtinen, S. J., Ibrahim, F., & Ouwehand, A. C. (2012). Genus *Lactobacillus*. *Lactic acid bacteria: Microbiological and functional aspects:* (Vol. 7). Taylor&Francis Group.

Bayane, A., Diawara, B., Dubois, R. D., Destain, J., Roblain, D., & Thonart, P. (2010). Isolation and characterization of new spore-forming lactic acid bacteria with prospects of use in food fermentations and probiotic preparations. *African Journal of Microbiology Research*, *4*, 1016–1025.

Berg, G. (2009). Plant-microbe interactions promoting plant growth and health: Perspectives for controlled use of microorganisms in agriculture. *Applied Microbiology and Biotechnology*, *84*(1), 11–18.

Bermudez-Brito, M., Plaza-Diaz, J., Munoz-Quezada, S., Gomez-Llorente, C., & Gil, A. (2012). Probiotic mechanisms of action. *Annals of Nutrition and Metabolism*, *61*(2), 160–174.

Bermúdez-Humarán, L. G., Motta, J. -P., Aubry, C., Kharrat, P., Rous-Martin, L., Sallenave, J. -M., et al. (2015). Serine protease inhibitors protect better than IL-10 and TGF-β anti-inflammatory cytokines against mouse colitis when delivered by recombinant lactococci. *Microbial Cell Factories*, *14*(1), 26.

Chalas, R., Janczarek, M., Bachanek, T., Mazur, E., Cieszko-Buk, M., & Szymanska, J. (2016). Characteristics of oral probiotics – A review. *Current Issues in Pharmacy and Medical Sciences*, *29*(1), 8–10.

Chandramouli, V., Kailasapathy, K., Peiris, P., & Jones, M. (2004). An improved method of microencapsulation and its evaluation to protect *Lactobacillus* spp. in simulated gastric conditions. *Journal of Microbiological Methods*, *56*(1), 27–35.

Chau, K., Lau, E., Greenberg, S., Jacobson, S., Yazdani-Brojeni, P., Verma, N., et al. (2015). Probiotics for infantile colic: A randomized, double-blind, placebo-controlled trial investigating *Lactobacillus reuteri* DSM 17938. *The Journal of Pediatrics*, *166*(1), 74–78. e1.

Chen, K. -N., Chen, M. -J., & Lin, C. -W. (2006). Optimal combination of the encapsulating materials for probiotic microcapsules and its experimental verification (R1). *Journal of Food Engineering*, *76*(3), 313–320.

Chichlowski, M., Croom, J., McBride, B., Havenstein, G., & Koci, M. (2007). Metabolic and physiological impact of probiotics or direct-fed-microbials on poultry: A brief review of current knowledge. *International Journal of Poultry Science*, *6*(10), 694–704.

Compant, S., Clément, C., & Sessitsch, A. (2010). Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry*, *42*(5), 669–678.

Cruz, A. G., Antunes, A. E., Sousa, A. L. O., Faria, J. A., & Saad, S. M. (2009). Ice-cream as a probiotic food carrier. *Food Research International*, *42*(9), 1233–1239.

da Silva, S. T., dos Santos, C. A., & Bressan, J. (2013). Intestinal microbiota; relevance to obesity and modulation by prebiotics and probiotics. *Nutricion Hospitalaria*, *28*(4), 1039–1048.

De Vleesschauwer, D., & Höfte, M. (2003). Using *Serratia plymuthica* to control fungal pathogens of plants. *Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, 2(460), 1–12.

De Vrese, M., & Schrezenmeir, J. (2008). Probiotics, prebiotics, and synbiotics. In *Food biotechnology* (pp. 1–66). Springer.

Deliza, R., Rosenthal, A., & Silva, A. (2003). Consumer attitude towards information on non conventional technology. *Trends in Food Science & Technology*, *14*(1–2), 43–49.

Dobson, A., Cotter, P. D., Ross, R. P., & Hill, C. (2012). Bacteriocin production: A probiotic trait? *Applied and Environmental Microbiology*, *78*(1), 1–6.

Doege, K., Grajecki, D., Zyriax, B. -C., Detinkina, E., zu Eulenburg, C., & Buhling, K. J. (2012). Impact of maternal supplementation with probiotics during pregnancy on atopic eczema in childhood–a meta-analysis. *British Journal of Nutrition*, *107*(1), 1–6.

Donkor, O., Henriksson, A., Vasiljevic, T., & Shah, N. (2006). Effect of acidification on the activity of probiotics in yoghurt during cold storage. *International Dairy Journal*, *16*(10), 1181–1189.

Doron, S., & Snydman, D. R. (2015). Risk and safety of probiotics. *Clinical Infectious Diseases*, *60*(Suppl. 2), S129–S134.

Duric, M. S., Ilicic, M. D., Milanovic, S. D., Caric, M. D., & Tekic, M. N. (2007). Nutritive characteristics of probiotic quark as influenced by type of starter. *Acta Periodica Technologica*, *38*, 11–19.

e Silva, J. P. S., & Freitas, A. C. (2014). In *Probiotic bacteria: Fundamentals, therapy, and technological aspects*. Taylor & Francis Group.

Fayol-Messaoudi, D., Berger, C. N., Coconnier-Polter, M. -H., Lievin-Le Moal, V., & Servin, A. L. (2005). pH-, Lactic acid-, and non-lactic acid-dependent activities of probiotic *Lactobacilli* against *Salmonella enterica* serovar *Typhimurium*. *Applied and Environmental Microbiology*, *71*(10), 6008–6013.

Gaggìa, F., Mattarelli, P., & Biavati, B. (2010). Probiotics and prebiotics in animal feeding for safe food production. *International Journal of Food Microbiology*, *141*, S15–S28.

Gorbach, S. (2002). Probiotics in the third millennium. *Digestive and Liver Disease*, *34*, S2–S7.

Gotcheva, V., Hristozova, E., Hristozova, T., Guo, M., Roshkova, Z., & Angelov, A. (2002). Assessment of potential probiotic properties of lactic acid bacteria and yeast strains. *Food Biotechnology*, *16*(3), 211–225.

Guandalini, S. (2011). Probiotics for prevention and treatment of diarrhea. *Journal of Clinical Gastroenterology*, *45*, S149–S153.

Guerra, P. V., Lima, L. N., Souza, T. C., Mazochi, V., Penna, F. J., Silva, A. M., et al. (2011). Pediatric functional constipation treatment with *Bifidobacterium*-containing yogurt: A crossover, double-blind, controlled trial. *World Journal of Gastroenterology: WJG*, *17*(34), 3916.

Hammes, W., & Hertel, C. (1998). New developments in meat starter cultures. *Meat Science*, *49*, S125–S138.

Hashemipour, H., Khaksar, V., & Kermanshahi, H. (2011). Application of probiotic on egg production and egg quality of chukar partridge. *African Journal of Biotechnology*, *10*(82), 19244–19248.

Haukioja, A. (2010). Probiotics and oral health. *European Journal of Dentistry*, *4*(03), 348–355.

Hekmat, S., & Reid, G. (2006). Sensory properties of probiotic yogurt is comparable to standard yogurt. *Nutrition Research*, *26*(4), 163–166.

Hirayama, K., & Rafter, J. (1999). The role of lactic acid bacteria in colon cancer prevention: Mechanistic considerations. *Antonie Van Leeuwenhoek*, *76*(1–4), 391–394.

Hotel, A. C. P., & Cordoba, A. (2001). Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. *Prevention*, *5*(1), 1–10.

Hsieh, P. S., Tsai, Y. C., Chen, Y. C., Teh, S. F., Ou, C. M., & King, V. A. E. (2012). Eradication of *Helicobacter pylori* infection by the probiotic strains *Lactobacillus johnsonii* MH-68 and *L. salivarius* ssp. *salicinius* AP-32. *Helicobacter*, *17*(6), 466–477.

Ibrahim, F., Ruvio, S., Granlund, L., Salminen, S., Viitanen, M., & Ouwehand, A. C. (2010). Probiotics and immunosenescence: Cheese as a carrier. *FEMS Immunology & Medical Microbiology*, *59*(1), 53–59.

Jørgensen, M. R., Kragelund, C., Jensen, P. Ø., Keller, M. K., & Twetman, S. (2017). Probiotic *Lactobacillus reuteri* has antifungal effects on oral *Candida* species in vitro. *Journal of Oral Microbiology*, *9*(1), 1274582.

Kaila, M., Isolauri, E., Soppi, E., Virtanen, E., Laine, S., & Arvilommi, H. (1992). Enhancement of the circulating antibody secreting cell response in human diarrhea by a human *Lactobacillus* strain. *Pediatric Research*, *32*(2), 141–144.

Kailasapathy, K., & Chin, J. (2000). Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. *Immunology and Cell Biology*, *78*(1), 80–88.

Kalavathy, R., Abdullah, N., Jalaludin, S., & Ho, Y. (2003). Effects of *Lactobacillus* cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. *British Poultry Science*, *44*(1), 139–144.

Kawakami, S. -I., Yamada, T., Nakanishi, N., & Cai, Y. (2010). Feeding of lactic acid bacteria and yeast on growth and diarrhea of *Holstein calves*. *Journal of Animal and Veterinary Advances*, *9*(7), 1112–1114.

Kedia, G., Wang, R., Patel, H., & Pandiella, S. S. (2007). Use of mixed cultures for the fermentation of cereal-based substrates with potential probiotic properties. *Process Biochemistry*, *42*(1), 65–70.

Kianifar, H., Ahanchian, H., Grover, Z., Jafari, S., Noorbakhsh, Z., Khakshour, A., et al. (2014). Synbiotic in the management of infantile colic: A randomised controlled trial. *Journal of Paediatrics and Child Health*, *50*(10), 801–805.

Kim, T. -S., Hur, J. -W., Yu, M. -A., Cheigh, C. -I., Kim, K. -N., Hwang, J. -K., et al. (2003). Antagonism of *Helicobacter pylori* by bacteriocins of lactic acid bacteria. *Journal of Food Protection*, *66*(1), 3–12.

Klaenhammer, T. R., Barrangou, R., Buck, B. L., Azcarate-Peril, M. A., & Altermann, E. (2005). Genomic features of lactic acid bacteria effecting bioprocessing and health. *FEMS Microbiology Reviews*, *29*(3), 393–409.

Krockel, L. (2006). Use of probiotic bacteria in meat products. *Fleischwirtschaft*, *86*, 109–113.

Langhout, P. (2000). New additives for broiler chickens. *World Poultry*, *16*(3), 22–27.

Lee, Y. -K., Puong, K. -Y., Ouwehand, A. C., & Salminen, S. (2003). Displacement of bacterial pathogens from mucus and Caco-2 cell surface by lactobacilli. *Journal of Medical Microbiology*, *52*(10), 925–930.

Leistner, L. (2000). Basic aspects of food preservation by hurdle technology. *International Journal of Food Microbiology*, *55*(1–3), 181–186.

Liu, X., Cao, S., & Zhang, X. (2015). Modulation of gut microbiota–brain axis by probiotics, prebiotics, and diet. *Journal of Agricultural and Food Chemistry*, *63*(36), 7885–7895.

Luckow, T., & Delahunty, C. (2004). Consumer acceptance of orange juice containing functional ingredients. *Food Research International*, *37*(8), 805–814.

Luckow, T., Sheehan, V., Fitzgerald, G., & Delahunty, C. (2006). Exposure, health information and flavour-masking strategies for improving the sensory quality of probiotic juice. *Appetite*, *47*(3), 315–323.

Mack, D., Ahrné, S., Hyde, L., Wei, S., & Hollingsworth, M. A. (2003). Extracellular MUC3 mucin secretion follows adherence of *Lactobacillus* strains to intestinal epithelial cells in vitro. *Gut*, *52*(6), 827–833.

MacNeil, J. D. (2005). The joint food and agriculture organization of the united nations/world health organization expert committee on food additives and its role in the evaluation of the safety of veterinary drug residues in foods. *The AAPS Journal*, *7*(2), E274–E280.

Malin, M., Suomalainen, H., Saxelin, M., & Isolauri, E. (1996). Promotion of IgA immune response in patients with Crohn's disease by oral bacteriotherapy with *Lactobacillus* GG. *Annals of Nutrition and Metabolism*, *40*(3), 137–145.

Martinez-Villaluenga, C., Frias, J., Gomez, R., & Vidal-Valverde, C. (2006). Influence of addition of raffinose family oligosaccharides on probiotic survival in fermented milk during refrigerated storage. *International Dairy Journal*, *16*(7), 768–774.

Mogna, L., Pane, M., Nicola, S., & Raiteri, E. (2014). Screening of different probiotic strains for their in vitro ability to metabolise oxalates: Any prospective use in humans? *Journal of Clinical Gastroenterology*, *48*, S91–S95.

Mousavi, Z., Mousavi, S., Razavi, S., Emam-Djomeh, Z., & Kiani, H. (2011). Fermentation of pomegranate juice by probiotic lactic acid bacteria. *World Journal of Microbiology and Biotechnology*, *27*(1), 123–128.

Ng, S., Hart, A., Kamm, M., Stagg, A., & Knight, S. C. (2009). Mechanisms of action of probiotics: Recent advances. *Inflammatory Bowel Diseases*, *15*(2), 300–310.

Ohashi, Y., & Ushida, K. (2009). Health-beneficial effects of probiotics: Its mode of action. *Animal Science Journal*, *80*(4), 361–371.

Oliveira, R. P., Florence, A. C., Silva, R. C., Perego, P., Converti, A., Gioielli, L. A., et al. (2009). Effect of different prebiotics on the fermentation kinetics, probiotic survival and fatty acids profiles in nonfat symbiotic fermented milk. *International Journal of Food Microbiology*, *128*(3), 467–472.

Özer, B. H., & Kirmaci, H. A. (2010). Functional milks and dairy beverages. *International Journal of Dairy Technology*, *63*(1), 1–15.

Paseephol, T., & Sherkat, F. (2009). Probiotic stability of yoghurts containing Jerusalem artichoke inulins during refrigerated storage. *Journal of Functional Foods*, *1*(3), 311–318.

Pereiraa, A. L. F., Maciela, T. C., & Rodriguesa, S. (2011). Probiotic cashew apple juice. *International Congress on Engineering and Food*, *2011*, 1–6.

Phavichitr, N., Puwdee, P., & Tantibhaedhyangkul, R. (2013). Cost-benefit analysis of the probiotic treatment of children hospitalized for acute diarrhea in Bangkok, Thailand. *The Southeast Asian Journal of Tropical Medicine and Public Health*, *44*(6), 1065–1071.

Phillips, M., Kailasapathy, K., & Tran, L. (2006). Viability of commercial probiotic cultures (*L. acidophilus*, *Bifidobacterium* sp., *L. casei*, *L. paracasei* and *L. rhamnosus*) in cheddar cheese. *International Journal of Food Microbiology*, *108*(2), 276–280.

Preston, G. M. (2004). Plant perceptions of plant growth-promoting *Pseudomonas*. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *359*(1446), 907–918.

Risoen, P., Rønning, P., Hegna, I., & Kolstø, A. B. (2004). Characterization of a broad range antimicrobial substance from *Bacillus cereus*. *Journal of Applied Microbiology*, *96*(4), 648–655.

Rodrigues, D., Rocha-Santos, T. A., Pereira, C. I., Gomes, A. M., Malcata, F. X., & Freitas, A. C. (2011). The potential effect of FOS and inulin upon probiotic bacterium performance in curdled milk matrices. *LWT—Food Science and Technology*, *44*(1), 100–108.

Rokka, S., & Rantamäki, P. (2010). Protecting probiotic bacteria by microencapsulation: Challenges for industrial applications. *European Food Research and Technology*, *231*(1), 1–12.

Ross, R., Fitzgerald, G., Collins, K., & Stanton, C. (2002). Cheese delivering biocultures—Probiotic cheese. *Australian Journal of Dairy Technology*, *57*(2), 71.

Rozada-Sánchez, R., Sattur, A. P., Thomas, K., & Pandiella, S. S. (2008). Evaluation of *Bifidobacterium* spp. for the production of a potentially probiotic malt-based beverage. *Process Biochemistry*, *43*(8), 848–854.

Saarela, M. (2009). Probiotics as ingredients in functional beverages. In *Functional and speciality beverage technology* (pp. 55–70). Amsterdam, the Netherlands: Elsevier.

Saarela, M., Mogensen, G., Fonden, R., Mättö, J., & Mattila-Sandholm, T. (2000). Probiotic bacteria: Safety, functional and technological properties. *Journal of Biotechnology*, *84*(3), 197–215.

Saarela, M., Virkajarvi, I., Alakomi, H. -L., Sigvart-Mattila, P., & Matto, J. (2006). Stability and functionality of freeze-dried probiotic *Bifidobacterium* cells during storage in juice and milk. *International Dairy Journal*, *16*(12), 1477–1482.

Sadeghzadeh, M., Rabieefar, A., Khoshnevisasl, P., Mousavinasab, N., & Eftekhari, K. (2014). The effect of probiotics on childhood constipation: A randomized controlled double blind clinical trial. *International Journal of Pediatrics*, *2014*, 1–6.

Sandoval-Castilla, O., Lobato-Calleros, C., García-Galindo, H., Alvarez-Ramírez, J., & Vernon-Carter, E. J. (2010). Textural properties of alginate–pectin beads and survivability of entrapped *L. casei* in simulated gastrointestinal conditions and in yoghurt. *Food Research International*, *43*(1), 111–117.

Servin, A. L. (2004). Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. *FEMS Microbiology Reviews*, *28*(4), 405–440.

Shah, N. (2000). Probiotic bacteria: Selective enumeration and survival in dairy foods. *Journal of Dairy Science*, *83*(4), 894–907.

Shah, N., Ding, W., Fallourd, M., & Leyer, G. (2010). Improving the stability of probiotic bacteria in model fruit juices using vitamins and antioxidants. *Journal of Food Science*, *75*(5), M278–M282.

Shah, N. P. (2007). Functional cultures and health benefits. *International Dairy Journal*, *17*(11), 1262–1277.

Sharifi Yazdi, M. K., Davoodabadi, A., Khesht Zarin, H. R., Tajabadi Ebrahimi, M., & Soltan Dallal, M. M. (2017). Characterisation and probiotic potential of lactic acid bacteria isolated from Iranian traditional yogurts. *Italian Journal of Animal Science*, *16*(2), 185–188.

Sheng, X. -F., Xia, J. -J., Jiang, C. -Y., He, L. -Y., & Qian, M. (2008). Characterization of heavy metal-resistant endophytic bacteria from rape (*Brassica napus*) roots and their potential in promoting the growth and lead accumulation of rape. *Environmental Pollution*, *156*(3), 1164–1170.

Siener, R., Bangen, U., Sidhu, H., Hönow, R., Von Unruh, G., & Hesse, A. (2013). The role of *Oxalobacter formigenes* colonization in calcium oxalate stone disease. *Kidney International*, *83*(6), 1144–1149.

Sinha, V., Vinay, A., & Bhinge, J. (2008). Nanocochleates: A novel drug delivery technology. *Pharma info Net*, *6*(5), 1918–5561.

Sozer, N., & Kokini, J. L. (2009). Nanotechnology and its applications in the food sector. *Trends in Biotechnology*, *27*(2), 82–89.

Suresh, K., Krishnappa, S., & Bhardwaj, P. (2013). Safety concerns of probiotic use: A review. *Safety*, *34*, 40.

Tan, T. P., Ba, Z., Sanders, M. E., D'Amico, F. J., Roberts, R. F., Smith, K. H., et al. (2017). Safety of *Bifidobacterium animalis* subsp. *lactis* (*B. lactis*) strain BB-12®-supplemented yogurt in healthy children. *Journal of Pediatric Gastroenterology and Nutrition*, *64*(2), 302.

Taverniti, V., Scabiosi, C., Arioli, S., Mora, D., & Guglielmetti, S. (2014). Short-term daily intake of 6 billion live probiotic cells can be insufficient in healthy adults to modulate the intestinal *bifidobacteria* and *Lactobacilli*. *Journal of Functional Foods*, *6*, 482–491.

Tillisch, K., Labus, J., Kilpatrick, L., Jiang, Z., Stains, J., Ebrat, B., et al. (2013). Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology*, *144*(7), 1394–1401. e4.

Työppönen, S., Petaja, E., & Mattila-Sandholm, T. (2003). Bioprotectives and probiotics for dry sausages. *International Journal of Food Microbiology*, *83*(3), 233–244.

Unno, Y., Okubo, K., Wasaki, J., Shinano, T., & Osaki, M. (2005). Plant growth promotion abilities and microscale bacterial dynamics in the rhizosphere of Lupin analysed by phytate utilization ability. *Environmental Microbiology*, *7*(3), 396–404.

Van Loon, L. (2007). Plant responses to plant growth-promoting rhizobacteria. In *New perspectives and approaches in plant growth-promoting Rhizobacteria research* (pp. 243–254). Springer.

Vandenplas, Y., Huys, G., & Daube, G. (2015). Probiotics: An update. *Jornal de Pediatria (Versão em portugues)*, *91*(1), 6–21.

Vasiljevic, T., & Shah, N. P. (2008). Probiotics-from Metchnikoff to bioactives. *International Dairy Journal*, *18*(7), 714–728.

Viaşu-Bolocan, L., Popescu, F., & Bica, C. (2013). Probiotics and their immunomodulatory potential. *Current Health Sciences Journal*, *39*, 204–209.

Vine, N., Leukes, W., Kaiser, H., Daya, S., Baxter, J., & Hecht, T. (2004). Competition for attachment of aquaculture candidate probiotic and pathogenic bacteria on fish intestinal mucus. *Journal of Fish Diseases*, *27*(6), 319–326.

Von Wright, A., & Axelsson, L. (2012). Lactic acid bacteria: An introduction. In S. Lahtinen, A. C. Ouwehand, S. Salminen, & A. von Wright (Eds.), *Lactic acid bacteria. Microbiological and functional aspects* (4th ed., pp. 1–14). Boca Raton, FL: Taylor & Francis Group.

Wang, C. -Y., Ng, C. -C., Su, H., Tzeng, W. -S., & Shyu, Y. -T. (2009). Probiotic potential of noni juice fermented with lactic acid bacteria and bifidobacteria. *International Journal of Food Sciences and Nutrition*, *60*(Suppl. 6), 98–106.

Wang, Y. -B. (2007). Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. *Aquaculture*, *269*(1–4), 259–264.

Wilson, E., Seo, C., Shahbazi, A., & Ibrahim, S. (2004). Survival and growth of probiotic cultures in sour cream products. In *IFT Annual Meeting. A*.

Wohlgemuth, S., Loh, G., & Blaut, M. (2010). Recent developments and perspectives in the investigation of probiotic effects. *International Journal of Medical Microbiology*, *300*(1), 3–10.

Yeo, S. K., & Liong, M. T. (2010). Effect of prebiotics on viability and growth characteristics of probiotics in soymilk. *Journal of the Science of Food and Agriculture*, *90*(2), 267–275.

Yeşilova, Y., Çalka, O., Akdeniz, N., & Berktaş, M. (2012). Effect of probiotics on the treatment of children with atopic dermatitis. *Annals of Dermatology*, *24*(2), 189–193.

Zhao, Y., Thilmony, R., Bender, C. L., Schaller, A., He, S. Y., & Howe, G. A. (2003). Virulence systems of *Pseudomonas syringae* pv. tomato promote bacterial speck disease in tomato by targeting the jasmonate signaling pathway. *The Plant Journal*, *36*(4), 485–499.

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Index

Note: Page numbers followed by "f" indicate figures, "t" indicate tables.

A

Ace (adhesin to collagen of *Enterococcus faecalis*), 29
Acetilactobacillus, 6
Acetyl-CoA acyltransferase 2 (Acaa2), 66
N-Acetylgalactosamine (GalNAc), 45
N-Acetylglucosamine (GlcNAc), 45
Acetyltransferase-2 (NAT-2), 195
Acm, Ebp (endocarditis- and biofilm-associated pili), 29
Actinobacteria, 327
  application as probiotics in livestock and poultry production, 359
  biochemical characterization of, 337t
  correlation coefficient analysis of physicochemical parameters versus population density, 335t
  DNA Isolation and 16S rRNA amplification, 331
  effect of antibiotic sensitivity assay by screening against shrimp pathogens, 342t
  identification of, 338t
  isolation of, 336f
  molecular characterization of Actinobacterial strain, 339
  morphological characteristics of Actinobacteria on SCA medium, 336t
  screening of antimicrobial activity against human pathogen, 337
  secondary screening of selected Actinobacteria against shrimp pathogens by well diffusion method, 341t
Adhesion ability, 27
Adhesion to gut cells, 26
ADME analysis, 123, 127
ADME properties of docked compounds by QikProp, 128t
Adrenocorticotropic hormone (ACTH), 142
Aeromonas hydrophila, 141, 261, 310
Agar-well diffusion assay, 5
Agaricus bisporus, 87t, 92, 93t
Aggregation-promoting factor (APF), 27
Agmatine, 262
Agricultural applications of probiotics, 526
Agrilactobacillus, 6
Agrocybe aegerita, 86
Akkermansia muciniphila, 14, 45, 47
  action, Possible mechanism of, 52
  characteristics, 48
  in host health, effects of, 48, 53f
    negative effects of, 54t
    positive effects of, 49t
  safety aspects of, 54
Allergy, 45
Alteromonas, 310
American Society for Microbiology (ASM) recommends techniques, 40
Amino acids and proteins, 262
Amylolactobacillus, 6
Angiopoietin-like protein 4/fasting-induced adipose factor (ANGPTL4/FIAF), 53
Antibiotic-associated diarrhea (AAD), 46
Antibiotics
  alternatives to the usage of, 351
  and bacteriocins, comparison of, 279t
  impact of antibiotic on the environment and consumer health, 350
  and microbial resistance, 350
  poultry production, exploitation of antibiotics in, 349
  resistance, 29
Anticancer properties, of probiotic foods, 175
Antiinflammatory activity, of probiotic food and human health, 174
Antimicrobial efficacy, 5
Antimicrobial peptides, 52
Antimicrobial potential of probiotic foods, 174
Antiobesity and probiotic foods, 175, 378
Antipathogenic activity, 27
Anxiety, 4
Apilactobacillus, 6
Aquaculture, 327
  use of probiotics in, 248
Aquaculture hatcheries, 247
2-Arachidonoylglycerol (2-AG), 52
Arginine decarboxylation, 262
AS (aggregation substance), 29
Associated 2-oleoylglycerol (2-OG), 52
Auricularia judae, 92
Autoimmunity, 45

B

Bacillus coagulans SNZ 1969, 13
Bacillus fragilis, 47
Bacillus subtilis C-3102, 13
Bacillus subtilis R0179, 13
Bacillus subtilis R0179, 13
Bacillus subtilis SNZ 1972, 13
Bacillus amyloliquefaciens, 286
Bacillus anthracis, 25, 234
Bacillus cereus, 25, 234
Bacillus clausii SNZ 1971, 13
Bacillus coagulans, 3, 234, 312, 314, 315, 317, 320
Bacillus probiotics, commercially available, 240, 241t
Bacillus probiotics, mechanism of action of, 239f, 239, 240f
Bacillus probiotics—safety, 242
Bacillus pseudomycoides, 25
Bacillus pyocyaneus., 311
Bacillus racemilacticus, 234
Bacillus sp. pathogenic attributes of, 241
Bacillus sp. probiotics, primary health benefits of, 235t
Bacillus spores, 234, 310
Bacillus subtilis, 3, 297
Bacillus subtilis BT23, 311
Bacillus synbiotics, primary beneficial effects of, 238t
Bacillus thuringiensis, 25, 234
Bacillus weihenstephanensis, 25
Bacteria commonly found in probiotic food products, 412t
Bacteria-derived blood metabolites, 208
Bacterial enzymatic activity, effects on, 190
Bacterial exopolysaccharides, 298
Bacterial populations in GI tract of poultry, 358
Bacteriocin, 27, 278, 281f, 282t, 321
  applications of, 285
    animal health, 286
    anticancer effect, 287
    antiulcerogenic effect, 288
    antiviral activity, 286
    application in foods, 285
    bacterial vaginosis, 288
    human health, 286
    spermicidal effect, 288
    urinary tract infections, 288
  from *Bacillus* species, 297
  biochemical characterization of, 295
  classification, 295
    Class I bacteriocins and their host producers, 282t
    Class II bacteriocins and their host producers, 282t
    Class II bacteriocins (unmodified peptides), 281

Bacteriocin (*Cont.*)
Class III bacteriocins (large proteins), 281
cytotoxicity effect of, 296
on different cancer cell lines, Effects of, 287t
genetics and biosynthesis, 295
from *Lactobacillus* sp., 296
mechanism of antibacterial activity of, 284
from probiotic strains, 294
Bacteroidales secreted antimicrobial protein-1 (BSAP-1), 58
Bacteroides fragilis, 14, 45, 55
characteristics, 55
effects in host health, 55, 56t, 57f
possible mechanism of action, 55
safety aspects of, 58
Bacteroides thetaiotaomicron, 14
Bacteroides xylanisolvens, 14
Beet juice, 171
Berendsen thermostat coupling, 123
Bifidobacteria animalis subsp. *animalis*, 24
Bifidobacteria animalis subsp. *lactis*, 24
Bifidobacteria breve, 24
Bifidobacteria longum subsp. *infantis*, 24
Bifidobacteria longum subsp. *Longum*, 24
Bifidobacterium adolescentis, 24
Bifidobacterium angulatum, 24
Bifidobacterium animalis subsp. *Lactis*, 11t
Bifidobacterium animalis ssp. *lactis* species, Bf26 and Bf141, 224
Bifidobacterium animalis subsp. *lactis* B94, 11
Bifidobacterium animalis subsp. *lactis* BB12, 11, 171
Bifidobacterium animalis subsp. *lactis* LKM512, 11
Bifidobacterium animalis subsp. *lactis*, 3
Bifidobacterium bifidum, 3, 11t, 24, 42, 91, 170
Bifidobacterium bifidum ATCC 29521, 12
Bifidobacterium bifidum BCMC 02290, 12
Bifidobacterium bifidum BF3, 12
Bifidobacterium bifidum CUL20, 12
Bifidobacterium bifidum IBRC-M10771, 12
Bifidobacterium bifidum R0071, 12
Bifidobacterium bifidum YIT 10347, 12
Bifidobacterium breve, 3, 11t, 24
Bifidobacterium breve A1, 12
Bifidobacterium breve Bb99, 12
Bifidobacterium breve Bif195, 12
Bifidobacterium breve BR3, 12
Bifidobacterium breve M-16V, B. breve B-3, 12
Bifidobacterium breve Yakult, 12
Bifidobacterium catenulatum, 24
Bifidobacterium dentium, 24
Bifidobacterium gallicum, 24
Bifidobacterium infantis BCMC 02129, 12
Bifidobacterium lactis 420, 11
Bifidobacterium lactis BB-12, 11
Bifidobacterium lactis CECT 8145, 11
Bifidobacterium lactis CNCC I-3446, 11
Bifidobacterium lactis CUL34, 11
Bifidobacterium lactis HN019, 11, 172
Bifidobacterium longum BCMC 02120, 12
Bifidobacterium longum BG7, 12
Bifidobacterium longum CECT 7347, 11
Bifidobacterium longum subsp. *infantis* BT1, 12
Bifidobacterium longum, 3, 11t, 24
Bifidobacterium pseudocatenulatum, 24
Bioactive compounds by probiotic microbes, Importance of, 173f
Biological control, 321
Biomaterials for probiotics encapsulation selection, 40
Biosafety, 109
Biosynthetic gene cluster analysis in Lactobacillus sp. using antiSMASH, 117t
Boletus erythropus, 92
Bombilactobacillus, 6
BopD (biofilm formation), 29

C

Cabbage juice, 171
CAGR (Compound Annual Growth Rate), 266
Calcium alginate, 130f
Calocybe indica, 92
Campbell-like integration, 105
Capsule formation, 29
Carbon disulfides, 150
Carcinogen/genotoxic compounds, Effects on, 190
Cardiovascular diseases (CVDs), 217
Carnitine palmitoyltransferase 1, 66
Carnobacterium divergens, 310
Carrageenan producing seaweed species, 251f
Carrageenophytes, cultivation and harvest of, 250f
Catecholamine, 142
cDNA (complementary DNA) libraries, 102
Cell survival during GIT transit, Significance of, 502
Cell survival during processing and storage, Significance of, 502
Central nervous system (CNS), 103
Cereal-based fermented foods, 457
rice-based food, 458
appam, 459
nutritional value, 459
therapeutic potential, 459
dosa, 458
microorganisms, 458
nutritional value, 459
therapeutic potential, 459
idli, 458
microorganisms, 458
nutritional value, 458
therapeutic potential, 458
Cereals and soya-based probiotic products, 171
Chitosan, 40
Chocolate-based probiotic products, 172
Christensenella minuta, 14
Chromosomal integration vectors, 104
Chronic inflammation, as a major oncogenic stimulant, 188
Clostridioides difficile–associated diarrhea, 4
Clostridium butyricum, 3
Clostridium butyricum CBM 588, 13
Clostridium butyricum CGMCC0313-1, 13
Clostridium butyricum MIYAIRI 588, 13
Clostridium perfringens, 142
Coaggregation activity, 27
Colicins, 279
Colorectal cancer, 154
Commercial food products containing prebiotics, widely available to consumers, 428t
fructans, 427
galactooligosaccharides, 428
lactulose, 429
miscellaneous oligosaccharides, 429
noncarbohydrates prebiotics, 429
resistant starch (RS) and glucose-derived oligosaccharides, 428
Commercial probiotics used in poultry, 354t
Commercial synbiotics: obstacles, challenges, and future prospects, 157
Companies involved in probiotic microbial formulation as feed additive for, 426t
Companilactobacillus, 6
Corticosterone, 142
Corynebacterium aureus, 398
Corynebacterium faecalis, 398
Corynebacterium glutamicum, 398
CRISPR/Cas systems, 106, 107
Crohn's disease (CD), 138, 152
Cryoprotectants, 39
Cryptic plasmids, 103, 104, 109
Cultivation parameters, 265
Culture media composition, 264
Cytokine levels, 152
Cytolysin, 29

D

Dairy-based probiotic foods, 169
cheese, 170
fresh milk and fermented milk products, 169
quark, chocolate mousse, frozen fermented dairy desserts, sour cream, and ice cream, 170
whey, 170
yogurt, 170
De Man, Rogosa, and Sharpe (MRS) broth, 4
Dellaglioa, 6
Denaturing gradient gel electrophoresis, 30
Dendritic cells, 62
Depression, 4, 210
Diarrheal diseases, 153
Dicentrarchus labrax, 256
DNA–DNA hybridization, 4, 5, 30
DNA methylation, 227
DNA transfer, 105
Docking score analysis, 124
Docking score and binding energy calculations of compounds, 125t
Dosa, 386
health benefits of, 387
Duodenum, 38
Dysbiosis, 265
types of, 222t

E

EcbA, EfaA, Esp (enterococcal surface protein), 29
Ecklonia maxima, 249
Edwardsiella, 310
EFSA (European Food Safety Authority), 24

ELISA tests, 5
Embden–Meyerhof–Parnas (EMP) pathway, 5
Encapsulation. *See also* Microencapsulation; Nanoencapsulation of probiotics
  applications, to deliver probiotics into foods, 489t
  methods, effective, 504
  of probiotics, 508
    industrial applications, 508
  strategies for postbiotics, 270t
  technologies for probiotics, 39
    for effective delivery of, 500
Enterococcus faecium, 3
Enterococcus faecium R0026, 13
Enterotoxin production, 242
Eosinophils, 188
EPS. *See* Exopolysaccharides (EPS)
Escherichia coli, 3, 163
Escherichia coli Nissle 1917, 13
E-test (Epsilometer Test), 29
Eubacterium hallii, 47, 64
  characteristics, 64
  effects in host health, 64, 64t, 65f
  possible mechanism on host, 65
  safety aspects of, 66
Eucheuma denticulatum, 250
Eucheuma denticulatum DOTY, 250, 251f
Eukaryotic-like ubiquitin protein (BfUbb) antagonizing strains, 58
European Food Safety Authority (EFSA), evaluate safety of foods and ingredients, 70
Exopolysaccharides (EPS), 278
  anticancer effect, 302
  antioxidant property, 302
  biological properties of bacterial EPS, 302
  biosynthesis and genetics of EPS, 300
  characteristics, 298
  cholesterol-lowering activity, 303
  classification, 298
  immunostimulatory activity, 302
  physicochemical properties of EPS, 301
  production, 301
    by probiotic bacteria, 299t
Expression systems, 106

F

Faecalibacterium prausnitzii, 14, 47, 58
  characteristics, 58
  effects in health, 59t
  effects in host health, 62, 63f
  possible mechanism of action, 62
  safety aspects of, 63
FDA GRAS guidelines, 28
Fecal microbiota transplantation (FMT), 46
Feed probiotics, 310
Fermented cereals microorganism, 412t
Fermented fish- and meat-based foods, 411t
Fermented food classification, based on different substrates, 450
  cheese, 453
    cheese making, Overview of, 453
    cottage cheese and soft cheese, 453
    nutritional value of, 454
    therapeutic role of, 455
  Kefir, 455
    anticancer activity of, 457
    antiinflammatory effect of, 457
    effects of kefir in gastrointestinal disorders, 456
    nutritional value of, 456
    preparation of, 455
    therapeutic potential of, 456
  yogurt, 450
    and gastrointestinal tract, 452
    nutritional value of, 451
    therapeutic role of, 452
Fermented vegetable food microorganism, 405
  Chinese and Korea kimchi microorganism, 406
  fermented legume-based foods microorganism, 408t
  FSP, fermented soy products microorganism, 407
  Korean soybean paste stew microorganism, 407
  Natto microorganism, 407
  serofluid dish microorganism, 406
  stinky tofu microorganism, 407
  tempeh microorganism, 407
Firmicutes, 24
Flammulina velutipes, 82, 92
Flippase (FLP)-dependent recombination, 105
Flow balance analysis (FBA), 108
Food Agricultural Organization (FAO), 3, 5, 23, 26, 40, 46, 68, 143, 242, 259, 277, 309, 349, 352, 382, 449, 500
  FAO/WHO guidelines for probiotics evaluation, 242f
Food applications of prebiotics, 443
Food-grade vectors, 103
Fructilactobacillus, 6
Fructo-oligosaccharide (FOS), 137
Fructophilic lactic acid bacteria, 14
Fruit- and vegetable-based probiotic products, 171
Fsr (E. faecalis regulator), 29
Fucose, 45
Functional food, 174, 177
Furfurilactobacillus, 6

G

β-Galactan, 130f
Galacto-oligosaccharide (GOS), 137
Galactose, 45
Gamma-aminobutyric acid (GABA), 205, 262
Ganoderma lucidum, 83f, 87t, 92, 93t
Gassericin from *Lactobacillus gasseri*, 297
Gastrointestinal (GI) tract, 38
  characteristics of the GI tract for probiotic delivery, 38
Geastrum saccatum, 92
Gelatinase, 29
GenBank, Selection of genes from, 114, 115
Genera of other probiotic bacteria and yeasts, 13
Generally recognized as safe (GRAS), 46
Genetic engineering applications on probiotic strains, 103
Genetic stability, 106
Genus *Bifidobacterium*, 10. *See also specific Bifidobacterium* sp.
GI tract issues, 212
Gibbs free energy, 131f
  landscape of MD trajectories, 131f
GLIDE docking protocol, 123
Global emergence of probiotic foods, 176
Glucagon-like peptides (GLP-1 and GLP-2), 65
G protein–coupled receptors (GPR43 and GPR41), 53, 65
Gram-negative bacteriocins, 279
Gram-positive bacteriocins, 280
Gut homeostasis, 47
Gut hormones, 208
Gut microbial irregularity, 212
Gut microbiome, 4
Gut microbiota
  in obesity, 222
  as psychobiotics, 204
  with psychotrophic properties, 205t

H

Helicobacter pylori, 163
Hepatic cytochrome P4501A2, 195
Hepatic encephalopathy (HE), 142
Hericium erinaceus, 92
Holzapfelia, 6
Human clinical trials of probiotic supplementation on overweight/obesity outcome, 225t
Human gastrointestinal tract (GIT) microflora, 24
Human health benefits of probiotics and postbiotics, 265
Human studies showing therapeutic effects of MDiet, 219t
Human symbionts, 45
Human TLR4/MD2 tetramer complex, 122
  with the Escherichia coli- LPS receptor structure, 122
Hyaluronidase, 29
Hydrogen bonds, 123, 127
Hydrogen peroxide, 174, 321

I

Ideal prebiotics, Characteristics of, 88
Idli, 383
  nutritional profile and health benefits, 383
  production of, 383
Ileum, 38
Immunofluorescence assays, 5
Immunomodulatory functions against cancer, 191
Industry-based probiotics application in various fields, 525
  cereal-based probiotic products, 526
  dairy-based probiotic foods, 525
  food applications of probiotics, 525
  fresh milk and fermented milks, 525
    dairy-based products, 525
    yogurt, 525

Industry-based probiotics application in various fields (*Cont.*)
fruit-based probiotic products, 526
meat-based probiotic foods, 526
Inflammatory bowel disease (IBD), 4, 45, 152
Innate immune system modulation, 121
Interaction site residues of all compounds with TLR4/MD2, 126t
Interleukin-8 (IL-8), 53
International Human Microbiome Consortium, 102
International Scientific Association for Probiotics and Prebiotics (ISAPP), 3, 204
Intervention strategies, 484
addition of protective ingredients, 488
encapsulation, 489, 489t
strain cultivation, 486
cross adaptation to stress, 487t
strain selection and inoculation condition, 484
Intramolecular hydrogen bond interactions
β-galactan, 129f
GSK1795091, 129f
Iota-carrageenan, 251f
Irritable bowel disease (IBS), 138
Irritable bowel syndrome, 4, 45, 154
Isochrysis sp., 248
Isomalto-oligosaccharides, 91

J

Jejunum, 38

K

Kanamycin esculin azide agar, 4
Kappa-carrageenan, 251f
Kappaphycus alvarezii Doty, 250, 251f
Kefir, 376
health benefits of, 377
anticancer properties, 377
antiobesity properties, 378
hypocholesterolemic properties, 378
probiotic value of, 376
production, 377
Ketones, 150
Kidney disease, 155
Kimchi, 367
health benefits of, 369
anticancer properties, 369
antioxidative effects, 370
hypolipidemic properties, 370
probiotic value of, 368
production of, 368
Kirby–Bauer disk-diffusion method, 29
Klebicins, 280
Kombucha, 373
biochemical profile of kombucha tea, 374
health benefits of, 375
anticancer properties, 375
antimicrobial properties, 375
antioxidant properties, 375
probiotic potential of, 374
Kyoto Gene and Genome Encyclopedia (KEGG), 102

L

Lactase concentration, 118f
Lactic acid, 321
Lactic acid bacteria (LAB), 23, 367
fermented food, 400t
with probiotic properties, 42
Lacticaseibacillus genus, 6, 8
Lacticaseibacillus casei (previously known as *L. casei*), 3, 8
Lacticaseibacillus paracasei (formerly known as *L. paracasei*), 8
Lacticaseibacillus rhamnosus (previously known as *L. rhamnosus*), 3, 8
Lactic corrosive microscopic organisms, 203
Lactiplantibacillus, 6, 8
Lactiplantibacillus plantarum, 3, 8t
Lactobacillus acidophilus, 3, 7, 7t, 24, 102, 170, 262, 312, 314, 315, 317, 320
Lactobacillus acidophilus ATCC 4356, 7
Lactobacillus acidophilus BCMC 12130, 7
Lactobacillus acidophilus CL1285, 7
Lactobacillus acidophilus CUL60 and CUL21, 7
Lactobacillus acidophilus GLA-14, 7
Lactobacillus acidophilus IBRC-M 10785, 7
Lactobacillus acidophilus LA-5, 7
Lactobacillus alimentarius, 24
Lactobacillus amylolyticus, 24
Lactobacillus amylovorus, 24
Lactobacillus animalis, 24
Lactobacillus aviaries, 24
Lactobacillus brevis, 24
Lactobacillus buchneri, 24, 262
Lactobacillus bulgaricus, 163
Lactobacillus bulgaricus OLL1073R-1, 7
Lactobacillus casei, 24
Lactobacillus casei BCMC 12313, 8
Lactobacillus casei CECT 9104, 8
Lactobacillus casei IBRC 10783, 8
Lactobacillus casei IBRC-M 10783, 8
Lactobacillus casei LBC80R, 8
Lactobacillus casei Shirota, 8, 27, 42
Lactobacillus casei var. *rhamnosus*, 8
Lactobacillus cellobiosus, 24
Lactobacillus collinoides, 24
Lactobacillus coryniformis, 24
Lactobacillus crispatus, 24
Lactobacillus curvatus, 24, 212
Lactobacillus delbrueckii, 6, 7, 24
Lactobacillus delbrueckii subsp. *Lactis*, 7t
Lactobacillus delbrueckii subsp. *Bulgaricus*, 3, 7t
Lactobacillus delbrueckii subsp. *Bulgaricus LBY-27*, 7
Lactobacillus diolivorans, 24
Lactobacillus farciminis, 24
Lactobacillus fermentum, 24
Lactobacillus fermentum GM-090, 10
Lactobacillus fermentum MCC2760, 174
Lactobacillus gallinarum, 24
Lactobacillus gasseri, 3, 7, 7t, 24, 297
Lactobacillus gasseri BRN17, 7
Lactobacillus gasseri CP2305, 7
Lactobacillus gasseri DSM 22583, 7
Lactobacillus gasseri PA-3, 7
Lactobacillus helveticus, 7, 7t, 24
Lactobacillus helveticus KLDS1.9204, 171
Lactobacillus helveticus R0052, 7
Lactobacillus hilgardii, 24
Lactobacillus jensenii, 7, 7t
Lactobacillus jensenii DSM 22567, 7
Lactobacillus johnsonii, 3, 7, 7t, 24
Lactobacillus kefiranofaciens, 24
Lactobacillus kefiri, 24
Lactobacillus lactis BCMC 12451, 7
Lactobacillus mucosae, 24
Lactobacillus panis, 24
Lactobacillus paracasei, 24, 156
Lactobacillus paracasei 8700:2, 8
Lactobacillus paracasei F19, 156
Lactobacillus paracasei GMNL-133, 8
Lactobacillus paracasei HP7, 8
Lactobacillus paracasei LBC-81, 171
Lactobacillus paracasei, L79;, 224
Lactobacillus paracasei SD-1, 8
Lactobacillus paracasei subsp. *paracasei* LBC82, 172
Lactobacillus paraplantarum, 24
Lactobacillus pentosus, 24
Lactobacillus plantarum, 24, 212, 249, 262
Lactobacillus plantarum 299v, 10, 171
Lactobacillus plantarum C29, 10
Lactobacillus plantarum CM53, 42
Lactobacillus plantarum DR7, 10
Lactobacillus plantarum F26., 156
Lactobacillus plantarum F5, 156
Lactobacillus plantarum HA119, 10
Lactobacillus plantarum HEAL9, 10
Lactobacillus plantarum IS-10506, 10
Lactobacillus plantarum, Lb38;, 224
Lactobacillus plantarum LRCC5193, 10
Lactobacillus plantarum P17630, 10
Lactobacillus plantarum PS128, 10
Lactobacillus plantarum TWK10, 10
Lactobacillus pontis, 24
Lactobacillus reuteri, 24
Lactobacillus reuteri 12246, 10
Lactobacillus reuteri ADR-1, 10
Lactobacillus reuteri ATCC PTA 5289, 10
Lactobacillus reuteri ATCC PTA6475, 10
Lactobacillus reuteri DSM 17938, 10
Lactobacillus reuteri RC-14, 10
Lactobacillus reuteri v3401, 10
Lactobacillus rhamnosus, 24, 91
Lactobacillus rhamnosus 19070-2, 9
Lactobacillus rhamnosus BMX 54, 9
Lactobacillus rhamnosus CGMCC 1.3724, 9
Lactobacillus rhamnosus CLR2, 9
Lactobacillus rhamnosus GG (LGG) ATCC 53103, 24
Lactobacillus rhamnosus GG, 9, 169
Lactobacillus rhamnosus GR-1, 9
Lactobacillus rhamnosus HN001, 9
Lactobacillus rhamnosus L8020, 9
Lactobacillus rhamnosus Lc705, 9
Lactobacillus rhamnosus, Lb102, 224
Lactobacillus rhamnosus SP1, 9
Lactobacillus sakei, 24
Lactobacillus salivarius, 24
Lactobacillus salivarius CECT 9145, 10

Lactobacillus salivarius PS7, 10
Lactobacillus sanfranciscensis, 24
Lactobacillus species, with GenBank accession IDs and genome size, 116t
Lactococcus lactis 11/19-B1, 13
Lactococcus lactis JCM 5805, 13
Lactococcus lactis N8, 171
Lactococcus lactis NRRL B-50571, 13
Lactococcus lactis subsp. *cremoris* FC, 13
Lactococcus lactis subsp. *lactis*, 3
Laminaria saccharina, 249
Lantibiotics, 285
Lapidilactobacillus, 6
Lates calcarifer, 253, 254
Latilactobacillus, 6
Lc. lactis/NICE (nisin-controlled expression) systems, 106
Legume-based foods, 459
  tempeh and miso, 459
    antioxidant potential, 461
    nutritional value and microbes, 460
    preparation of, 459
    therapeutic potential, 460
      role in gut health, 460
      of soy isoflavones, 461
Lentilactobacillus, 6
Lentinus edodes, 92
Leuconostoc mesenteroides, 3, 156
Leukocytes, 188
Levilactobacillus, 6
Ligand interactions diagram of top polysaccharides with TLR4/MD2, 126f
Ligilactobacillus, 6, 8
Ligilactobacillus salivarius, 3, 8t
Limosilactobacillus, 6, 8
Limosilactobacillus fermentum, 9t
Limosilactobacillus reuteri, 3, 9t
Lipopolysaccharides (LPS), 121, 151
Liquorilactobacillus, 6
Litopenaeus stylirostris, 322
Litopenaeus vannamei, 312, 314, 315t, 315, 316t, 317, 319t, 320
  analysis of water physiochemical parameters culture tank, 343t
  biochemical changes in tissue of, 345t
  immunological activity of, 346t
Live biotherapeutic products (LBPs), 3, 46, 69
Liver disease, 155
Livestock applications of probiotics, 527
Loigolactobacillus, 6
Lychee (*Litchi chinensis*), 25
Lymphocytes, 191

M

Macrophages, 62
MALDI–TOF technique, 5
Mangosteen (*Garcinia mangostana*), 25
Mangroves, 327
Mangrove soil samples, 329
  antimicrobial activity, determination of, 330
    agar well diffusion method, 331
    test microbes, 330
  isolation and identification of Actinobacteria, 329, 334
    from Muthupet mangrove soil samples, 334t
  physicochemical parameters, analysis of, 329, 333, 333t
    electrical conductivity and cation exchange capacity, 334
    macronutrients, 334
    micronutrients, 334
    organic carbon and organic matter, 334
    pH, 333
    soil texture, 333
  potential Actinobacteria, molecular characterization of, 331
    DNA Isolation and 16S rRNA amplification, 331
Mannose phosphotransferase system (Man-PTS), 285
Marketed prebiotics, 444t
Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, 30
Meat-based probiotic products, 172
Mechanism of prebiotics, 88
Metabolic disorder, 211
Metabolic flow analysis (MFA), 108
Metabolic syndrome (MetS), 152, 217, 218
  and diet, 218
Metabolic trace path analysis (MPA), 108
Methyl acetates, 150
MetS. *See* Metabolic syndrome (MetS)
Microbes–brain signalling, 206
Microbes immune interactions, 209
Microbes used as animal probiotics, 352
Microbial diversity, 327
Microbial species as probiotics, 164f
Microbiota of adult GI tract, 38
Microbiota and possibilities for treatment, regulation of, 212
Microcins, 279
Microencapsulation of probiotics, 503
  efficient matrices for, 503
  selecting biomaterials for, 40
Microorganism in fermented dairy products, 410
  cheese, 410
  yogurt, 410
Microorganism in fermented grain –based foods, 410
Mind-enteric nervous system interaction, 207
Miso, 384
  nutritional profile and health benefits, 384
  production of, 384
Molecular docking, 123, 124
Molecular dynamics, 123, 127
Monocyte chemo attractant protein-1 (MCP-1) expression, 53
Mucoadhesion, 38
Mushrooms
  bioactive compounds of, 84
  energy value of, 83
  as food values, 83
  in the gut, activity of, 93t
  importance of prebiotics, 86
  in India, 84
  low-molecular and high-molecular weight compounds in, 84
  as prebiotics, role of, 92
  as promising prebiotic, 90
  supplementations as a prebiotic, 82
  worldwide production of, 84t
Myeloid differentiation factor 2 (MD2), 121

N

Nannochloropsis, 253, 255f
Nanocapsulation in probiotics, advantages of, 529f
Nanoencapsulation of probiotics, 506
  biopolymer-based nanocarriers, 508
  lipid-based nanocarriers, 507
  nature-inspired nanocarriers, 507
  special equipment–based nanocarriers, 507
Neuropsychological disorders, 210
Neurotransmitters, 263
New dietary ingredient (NDI), 70
Next-generation probiotics (NGP), 14, 24, 46
  application of, 69
    current development, 69
    regulatory challenges, 70
    technical challenges, 69
  candidates for, 47
  safety assessment of, 68
NGP. *See* Next-generation probiotics (NGP)
Nondairy probiotic products, 171
  food products, 168t
Nonribosomal peptide synthetases (NRPSs), 103
Novel *Lactobacilli* genera, 6
Novel or next-generation probiotics, 14
nsr gene, 104
Nuclear factor-κB (NF-κB), 52
Nutraceutical importance, of probiotic foods, 177

O

Obesity, 222, 223, 225t. *See also* Antiobesity
Oreochromis niloticus, 321
Ortholog Group Clusters (COG), 102
Oxidative stress, modulation of, 189

P

Para-aminobenzoic acid (pABA), 264
Parabacteroides goldsteinii, 14, 47, 66
  characteristics, 66
  effects in host health, 66, 67t, 68f
  possible mechanism of action, 66
  safety aspects of, 68
Paralactobacillus, 6
Paralichthys olivaceus, 256
Paraprobiotic, 3
Particle size of seaweed thalli during the start of fermentation, 253f
Pasteurella., 310
Paucilactobacillus, 6
Pavlova sp., 248
Pediococcus acidilactici, 3
Pediococcus acidilactici MA 18/5, 322
Pediococcus pentosaceus, 156
Penaeus monodon, 311
Penaeus vannamei, 322
Peptidoglycan, 137

Peripheral blood mononuclear cell (PBMC), 62
Peroxisome proliferator–activated receptor gamma (PPARγ), 53
pH concentration, 115, 118
Phagocytosis, 38
Pharmacodynamic (PD), 127
Pharmacokinetic (PK), 127
Phosphoenolpyruvate (PEP), 262
Phylogenetic analysis of genes, 115, 117
Phylogenetic tree of lactase concentration in *Lactobacillus* sp. by using MEGA X, 118f
Phylogenetic tree of polyketide synthase (PKS) concentration in Lactobacillus sp. by MEGA X, 118f
Pichia kluyveri CCMA0615, 171
Pickles, 381
  health benefits of, 382
  production of, 382
Pinctada martensii, 249
pLEB-derived plasmids, 106
Pleurotus ostreatus, 92
Polyketide synthase (PKS) concentration, 118f
Polyketide synthase (PKS) genes, 103
Pond probiotics, 310
Porto-fundamental encephalopathy (PSE), 210
Postbiotic, 3, 247
  classification, 261
Poultry industry, 349
Poultry, key functions of probiotics in, 357f
Poultry, role of probiotics in, 356
Prebiotics, 3, 449
  as anticarcinogenic agents, mechanism of action of, 139
  benefits of, 87
    and potential mechanisms of action, 89t
  concept, 86
  criteria of, 92
  derived from various sources, 87t
  dietary fiber sources, 438
    arabinooligosaccharides, 439
    beta-glucan, 438
    GOSs, 438
    guar gum, 438
    inulin, oligofructose, and FOSs, 438
    isomaltooligosaccharides, 438
    lactulose, 438
    RS and maltodextrin, 438
    xylooligosaccharides, 439
  in foods, functional properties of, 442t
  food products, development of, 439
  formulations intended for livestock, 427t
  gastrointestinal effects of, 89, 90
  in immunomodulation, mechanism of action of, 139
  in improving nutritional absorption, mechanism of, 141
  in maintaining
    health against various diseases, 138f
    intestinal pH, Mechanism of action of, 138
    lipid metabolism, Mechanism of action of, 138
    nervous system, Mechanism of action of, 142
  index (PI), 86
  potential immunomodulatory mechanism of, 89
  in preventing
    autism, Mechanism of action of, 142
    bowel diseases, Mechanism of action of, 140
    cardiovascular diseases, Mechanism of action of, 143
    diabetes, Mechanism of action of, 140
    hepatic encephalopathy, Mechanism of action of, 142
    necrotizing enterocolitis (NEC), Mechanism of action of, 140
    skin diseases, Mechanism of action of, 143
  production from food industry wastes and agricultural by-products, 439
  properties of, 87
  for psychobiotics, 205
  safety, 442
  showing protective effect on gastrointestinal system, 138f
  in treating constipation, mechanism of, 138
  used in human and animal nutrition, 427
Prevotella copri, 14
Primary antibiotics and extracellular enzymes, produced by *Bacillus* probiotics, 236t
Principal component analysis of compounds obtained by MD trajectory, 131t
Probioactive perception, 293
Probioactives sources, and strain specificity of, 294
Probiotic attributes of *Bacillus* species, 234
Probiotic cultures, 24
Probiotic derivatives on TLR4/MD2, binding efficiency of, 122
Probiotic foods, 164, 168t
  and antidiabetic activities, 175
  clinical significance—human health perspectives, 174
  criteria for selection of, 450
  of different categories, 167f
  effect on brain and CNS, 175
  future perspectives of, 177
  on the market, 449
  and nutritional properties, Microbial role in, 172
  products, 167
    of various region, 166t
  production of, 367
    dosa, 386
    idli, 383
    kefir, 376
    kimchi, 367
    kombucha, 373
    miso, 384
    pickles, 381
    sauerkraut, 379
    tempeh, 370
    yoghurt, 384
  types of, 450
Probiotic fortifies seaweed silage, of *Eucheuma denticulatum* Doty, 250
Probiotic microorganisms, 24, 310
  detection methods and assays of, 4
    clinical studies, 6
    in vitro assays, 4
    in vivo animal assays, 6
  mostly intended for poultry and other livestock, 353t
  and recently reported health effects, 6
  used in animal nutrition, 423, 424t
    Lactobacillus, 424
    Bifidobacterium, 424
    Bacillus, 425
    Saccharomyces, 425
  used in the fermented food, 398
    acetobacter, 398
    lactic acid bacteria, 398
    mold, 404
      aspergillus, 405
      geotrichum, 405
      mucor, 405
      rhizopus, 405
    nonpathogenic *Corynebacterium*, 398
    yeast, 399
      cover up the odor and light salt effect, 404
      flavor intensity, 404
      improve flavour, 404
      increase nutrition, 404
      loose product, 404
      making noodles, 403
      protect the liver, 404
  used in human nutrition and their role, 418
    Bifidobacterium species, 421
      Bifidobacterium animalis ssp. *lactis* BB-12, 422
      Bifidobacterium breve Yakult, 422
      Bifidobacterium lactis HN019, 421
      Bifidobacterium longum BB536, 422
    Lactobacillus species, 418
      Lactobacillus acidophilus LA-5, 418
      Lactobacillus acidophilus NCDO 1748, 419
      Lactobacillus acidophilus NCFM, 419
      Lactobacillus casei Shirota, 419
      Lactobacillus gasseri OLL2716 (LG21), 420
      Lactobacillus paracasei ssp. *paracasei* F19, 420
      Lactobacillus reuteri RC-14, 421
      Lactobacillus rhamnosus GG (LGG), 420
      Lactobacillus rhamnosus GR-1, 420
      Lactobacillus rhamnosus HN001, 421
    Yeasts
      S. cerevisiae var. boulardii, 423
      Saccharomyces boulardii, 423
  and their recently reported health effects, 6
Probiotic polysaccharides and their assistant compounds, 122t
Probiotic species, 3, 519t. *See also* Probiotic microorganisms
Probiotic strains
  in emended genus *Lactobacillus*, positive health effects, reported between 2018 and 2020, 7, 7t
  requirements for selection of, 25
  selection, for technological performance, 499
    acid tolerance, 499

bile acid tolerance, 500
oxygen tolerance, 499
temperature tolerance, 500
Probiotics, 309, 449, 498
on apoptosis, effects of, 191
application of, 320
application challenges, 527
sensory acceptance, 528
viability and survival, 527
in aquaculture
competitive exclusion, 311
mechanisms of, 310
nutrient and enzymatic contributions to digestion, 311
assessment of growth performance, 313
assessment of physicochemical parameters, 313
and its beneficial effect, 521t
in aquaculture, 322t
biochemical analysis of *Litopenaeus vannamei* by the effect of potential bacteria in different intervals, 317, 319t, 320t
bioremediation, 311
against cancer, mechanism of action of, 189
on cancer, in vitro studies of, 192, 193t
on cancer, in vivo studies of, 193, 194t
characteristics of, 520, 520f
against colorectal cancer (CRC), 195
and cardiovascular diseases, 227
definition, concepts, and history, 352
definition and principles, 328, 498
in dietary supplements, 176
and dysbiosis, 222
effect of probiotic supplements on the *Litopenaeus vannamei* and analysis of physicochemical parameters, 315t, 316t
effect of reproduction of aquatic species, 322
feed preparation, 312
tank culture experiments, 312
in fermented food, function and characteristics of, 413t
in food fermentation, role of, 366
future perspective, 528
against gastric cancer (GC), 195
and gastrointestinal (GI) cancer, 195
and the genus *Bifidobacterium*, 10
growth and development of characteristics and survival of *Litopenaeus vannamei* culture tank with, 318t
health benefits of, 498, 520, 522f
hydrogen peroxide, 321
influence of immune system, 321
main functions of, 260t
microorganisms. *See* Probiotic microorganisms
mode of action of, 277
modulation of gut–brain axis by, 114
and obesity, 223
animal studies, 224
clinical trials, 225
physicochemical parameters of probiotics supplemented shrimp aquaculture tank, determination of, 314
and postbiotics with beneficial human health effects, 267t
and postbiotics for healthy food development, Application of, 266
for poultry nutrition, 354
to produce postbiotics, Conditions of, 264
putative mechanisms of, 165t
regulations and guidelines for, 529
harmful side effects of, 529
safety aspects of, 529
requirements demanded for, 260t
selection of, 355
antipathogenic activity, 356
colonization ability, 355
host-associated functional effects, 356
industrial requirements and technological attributes, 356
origin of the probiotic strain, 355
safety assessment, 355
stress tolerance ability, 355
synthesis of functional molecules, 356
on tumor microenvironment, Effects of, 191
types of, 309
feed probiotics, 310
pond probiotics, 310
unveiling the potential of, 221
viability, factors affecting, 481
competition with starter cultures, 483
food matrix features, 481
GIT transit, 484
processing conditions, 483
storage conditions, 484
in vitro and in vivo studies on beneficial effects of, 114
bowel diseases and immune system, 114
dental caries, 114
dermal health, 114
water quality improvement, 311
Propionibacterium freudenreichii subsp. *shermanii* JS, 13
Protein transport, 107
Protein-rich foods, 83
Pseudoalteromonas, 310
Pseudomonas sp., 310
Pseudonocardia alaniniphila
phylogenetic relationship of, 340f
secondary prediction of, 340f
Psychobiotics
psychophysiological effects of, 205
used in neurological conditions, 206t
PubMed database, 6, 7
Pyocins, 280

Q

Qualified presumption of safety (QPS), 24, 70, 353, 417, 424
recommendation for assessment of probiotic cultures, 28
Quantitative structure–activity relationships (QSAR), 108
3D-QSAR3D technologies, 108

R

Reactive oxygen species (ROS), 188
Real-time PCR, 5
Regenerating islet-derived 3-gamma (RegIIIγ), 52
Regulatory T (Treg) cells, 52
RMSD deviations of compounds generated by MD trajectory
calcium alginate, 129f
β-galactan, 129f
ROS. *See* Reactive oxygen species (ROS)
Roseobacter sp., 310
Ruditapes decussatus, 249

S

Saccharomyces boulardii, 3, 25, 278
Saccharomyces boulardii CNCM I-745, 13
Saccharomyces boulardii DBVPG, 13
Saccharomyces boulardii SAA655, 171
Saccharomyces cerevisiae, 25, 249
Saccharomyces cerevisiae CCMA0731, 171
Saccharomyces cerevisiae CCMA0732, 171
Saccharomyces cerevisiae var. *boulardii*, 13
Safety aspects of *Bacteroides fragilis*, 58
Safety assessments, 28
Safety evaluation criteria, 24
Salicylic acid, 63
Sauerkraut, 379
health benefits of, 380
ability to protect oxidative DNA damage, 381
anticarcinogenic activity, 381
antiinflammatory effects, 381
antioxidant properties, 381
nutritional profile of, 380
production of, 379
SCFA. *See* Short-chain fatty acids (SCFA)
Schizophyllan, 86
Schleiferilactobacillus, 6
Scm (second collagen adhesin of Enterococcus faecium), 29
Scrutiny of dataset, 115
Seaweed probiotic fermentation, 249
Seaweed silage as rotifer feed, 253
Seaweed silage feed formulation, 254
Seaweed silage, chemical characteristics of, 253
Secondary metabolite clusters identification using antiSMASH, 115, 116
Secundilactobacillus, 6
Selecting the in vitro conditions, for cells release, 41
Sensory characters improvement and limitations, 503
Sequence analyses, 102
Serine protease, 29
SgrA (serine-glutamate repeat A), 29
Short-chain fatty acids (SCFA), 58, 69, 91, 137, 143, 150, 151, 189, 191, 208, 261, 429, 442, 498
Shrimp diseases, 328
Shrimp pathogen activity, 337
Shrimp pathogen, control of, 328
Single nucleotide polymorphism variants, 102
Sirtuin-1 (SIRT1), 66
Spore forming probiotics (SFP), 239
Sporeformers in gut and food chain, Advantages of, 234

Spray-drying technique, 39
Streptococcus salivarius subsp. *thermophilus* *bacteria*, 170
Streptococcus thermophilus, 3
Streptococcus thermophilus MG510, 13
Streptococcus thermophilus STY-31, 13
Streptococcus thermophilus TH-4, 13
Stress tolerance, 322
Subtilosin A from Bacillus subtilis, 297
Survival during gastrointestinal transit, 26
Survival of entrapped LCS in simulated gastrointestinal conditions, 42
Symbiotic, 149
Synbiotics, 3, 450
  for animals, 155
  application of, 156
  of *Bacillus* sp., 237
  effect and its mechanism of action, 151f
  formation study using probiotic and prebiotic components, 157t
  for humans, 151
  mechanism of action of, 150
  and their outcomes on human health in clinical studies, 152
  rationale to use, 237
  safety issue of, 158
  selection criteria, 150
  therapy, 156
  in use, 150
  Yoghurt, 157
Systems biology approaches, 108

T

Tailocins, 280
Taxonomy/Identification, 30
Techno-functional traits approaches of probiotics, 522
  adhesion properties, 523
  antagonistic properties, 523
  anti-inflammatory properties, 524
  antimutagenic and anticarcinogenic properties, 524
  immunomodulatory properties, 524
  improved barrier function, 524
  probiotics, functional aspects of, 523f, 523
Technological requirements, 30
Technological selection criteria of probiotic strains for assessment of safety, 31
Tempeh, 370
  health benefits of, 372
    anticancer properties, 372
    antineurodegenerative properties, 373
    antioxidant properties, 372
    hypolipidemic properties, 372
  manufacture of, 371
Thalassiosira weissglogii, 248
TLR4/MD2 complex, 121
TLR4/MD2 signaling receptors, 122, 127
Toll-like receptors (TLRs), 52, 121
Transcription factor A (TFAM), 66
Trans-galacto-oligosaccharide, 137
Type 2 diabetes mellitus (T2DM), 217
Tyrosine hydroxylase, 142

U

Ulcerative colitis (UC), 152
Ulva reticulata, 249
Undaria pinnatifida, 249
Upper gastrointestinal (GI) intestinal tract diseases, prevention of, 37
Upper respiratory complaints, 163

V

Vagal signalling, 207
Vegetable- and fruit-based food, 461
  Kimchi, 465
    antimicrobial potential of LAB found in kimchi, 468
    antimutagenic and anticancer effect, 467
    antiobesity effect, 468
    antioxidant and antiaging potential, 467
    fermentation and microbes, 466
    nutritional value and therapeutic potential of, 466
    preparation, 466
    risks of kimchi consumption, 468
  kombucha, 461
    antidiabetic and anticancer potential, 463
    antimicrobial and antioxidant potential, 463
    beneficial microbes present in, 462
    detoxification potential, 463
    health benefits of tea, 462
    nutritive value and therapeutic potential of, 462
    potential for cardiovascular disease prevention, 463
    potential in improving mental health and stability, 463
    preparation, 461
    risks involved in kombucha consumption, 463
  pickles, 463
    beedana pickle, 465
    biochemistry of vegetable fermentations, 464
    chukh, 465
    cucumber fermentations, 464
    dheu pickle, 465
    elon pickle, 465
    kachalu pickle, 465
    kachnar pickle, 465
    lasura pickle, 465
    lingri pickle, 465
    nashpati (pear) pickle, 465
    olive fermentations, 464
    tamatar pickle, 465
  sauerkraut, 469
    anticancer and antioxidant potential, 469
    antimicrobial effect and gut health, 469
    detoxification, 469
    nutritional value of sauerkraut, 469
    preparation of, 469
    risks of sauerkraut consumption, 470
    therapeutic potential of, 469
Vibrio anguillarum, 310
Vibrio harveyi, 310
Virulence factors, 29
Virulence genes, 5
Vitamins, 9, 31, 48, 69, 83, 143, 155, 157, 163, 221, 240, 260t, 263, 302, 313, 353, 369, 380, 404, 440, 451, 454, 481, 506, 521
Voges–Proskauer test, 5

W

Western blotting analysis, 5
Whole-genome sequencing (WGS) of probiotic microorganisms, 5, 102
World Health Organization (WHO), 3, 5, 23, 40, 46, 217, 242, 259, 277, 309, 349, 352, 365, 379, 449, 500
Wound healing, 4

X

Xylooligosaccharides, 91

Y

Yeasts, 13, 24, 25, 37, 46, 101, 113, 163, 171, 221, 249, 256, 259, 310, 337, 353, 366, 374, 397, 399, 423, 455, 502, 527. *See also Saccharomyces* sp.
Yoghurt, 157, 384
  nutritional profile and health benefits, 386
  production of, 385

Z

Zonula occludens-1
Zonulin, 152
Zymetin, 321

ADVANCES IN PROBIOTICS

MICROORGANISMS IN FOOD AND HEALTH

Understand the latest research development of probiotics for health and disease safety in humans and animals

Advances in Probiotics: Microorganisms in Food and Health highlights recent advances in probiotic microorganisms, commercial probiotics, safety aspects of probiotics, preparation and commercialization, microbiome therapy for diseases and disorders, and next generation probiotics. This is a comprehensive resource of developments of new formulations and products for probiotic and prebiotic food with focus on the microorganisms to enable effective probiotic delivery. The book deliberates contemporary trends and challenges, risks, limitations in probiotic and prebiotic food to deliver an understanding not only for research development purposes but also to benefit further standardize industrial requirements and other techno-functional traits of probiotics.

At present there is no solitary volume to describe the probiotics and prebiotics properties, ***Advances in Probiotics: Microorganisms in Food and Health*** provides novel information to fill the overall gap in the market. It presents the most current information on probiotic and prebiotics for the food industry. This book is a valuable resource for academicians, researchers, food industrialists, and entrepreneurs.

- Presents a simulated gastrointestinal system to analyze the probiotics effects on gut microbiome for learning purpose
- Includes research information on Next Generation Probiotics to foster new formulations
- Provides comprehensive information on probiotic microorganism behavior for more accurate analysis
- Discusses the potential of probiotic and prebiotic foods in preventing disease

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An imprint of Elsevier
elsevier.com/books-and-journals

ISBN 978-0-12-822909-5