Socioeconomic Study of Prospective of Probiotics, Prebiotics, and Synbiotics for Sustainable Development of Aquaculture in Indian Sundarbans

Chapter · March 2022

DOI: 10.1007/978-981-16-8990-1_13

Swagat Ghosh
Ramakrishna Mission Vivekananda Educational Research Institute

Kambaska Kumar Behera
Renu Bist
Sangita Mohanty
Manojit Bhattacharya *Editors*

Prebiotics, Probiotics and Nutraceuticals

Springer

Prebiotics, Probiotics and Nutraceuticals

Kambaska Kumar Behera • Renu Bist •
Sangita Mohanty • Manojit Bhattacharya
Editors

Prebiotics, Probiotics and Nutraceuticals

Springer

Editors
Kambaska Kumar Behera
Fakir Mohan University, Vyasa Vihar
Balasore, Odisha, India

Renu Bist
Department of Zoology
University of Rajasthan
Jaipur, Rajasthan, India

Sangita Mohanty
ICAR-National Rice Research Institute,
Bidyadharpur
Cuttack, Odisha, India

Manojit Bhattacharya
Department of Zoology
Fakir Mohan University, Vyasa Vihar
Balasore, Odisha, India

ISBN 978-981-16-8989-5 ISBN 978-981-16-8990-1 (eBook)
https://doi.org/10.1007/978-981-16-8990-1

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Dedicated
To
ALL COVID WARRIORS
OF THE WORLD

Foreword

It gives me immense pleasure to write a foreword to the book *Prebiotics, Probiotics and Nutraceuticals* edited by Dr. Kambaska Kumar Behera (Fakir Mohan University, Balasore, Odisha, India) and his collaborators (Dr. Renu Bist (Rajasthan University, Rajasthan, India), Dr. Sangita Mohanty (ICAR-NRRI, Cuttack, Odisha, India) and Dr. Manojit Bhattacharya (Fakir Mohan University)). The book edited by this young and dynamic research group has attempted to cover all emerging fields of knowledge in the era of climate change with a special focus on Covid-19. As technologies develop, many conventional problems of food, environment and health have been alleviated. However, the continuous increase in the global population and the current scenario of the pandemic have been providing a screaming alert on global immunity to the future. It is true that technology revolution has contributed greatly to improve the quality of life, but it is also obvious that world pandemic issues have emerged from technology exploration. In this context, the research ethos has been increasing among the scientific community for providing bioactive compounds as an immune stimulant to enhance immunity to various diseases among the human population. The knowledge generation and/or exploration and application under this particular subject is huge and highly diversified as compared to other emerging scientific-cum-technological domain. To prepare and present for the young generation of specialized readers, the title *Prebiotics, Probiotics and Nutraceuticals* seems to be bold and mega-sized, and also exploratory. Encouragingly, Dr. Behera, other editor(s) and author(s) have been successful in touching upon almost all perceivable title points, to give 'completeness' to the volume.

The contributors of this book are mainly from reputed academic and scientific institutions across India. This book shall be considered as one of the best knowledge sources on the present status, trends and approaches of nutrition and food supplements. This volume is unique in providing practical knowledge and ideas in employing nutrition for human health to fight against pandemics and shall be used as a text for students, researchers, experts and policy makers. I hope this book

Prebiotics, Probiotics and Nutraceuticals will contribute a lot to the development of ideas on balancing sustainability and utility in the field of Food and Nutritional Biotechnology.

Uttam Kumar Sahoo

School of Earth Sciences & Natural Resource
Management, Mizoram (Central) University
Aizawl, Mizoram, India

Preface

The book *Prebiotics, Probiotics and Nutraceuticals* accelerates the molecular path of whole or parts of living organisms to produce or improve the immune system and processes to fight against diseases and specially sudden pandemics. It is a rapidly evolving branch of natural sciences which started with the creation of the first recombinant gene, 30 years ago in the field of molecular farming. These nutritional supplements are used in different ways, changing the way we live by improving the foods we eat, the beverages we drink and the medicines we take. They also have enhanced other aspects of our lives through the intake of Prebiotics, Probiotics and Nutraceuticals to fight many dreadful diseases such as arteriosclerosis, cancer, diabetes, Parkinson's and Alzheimer's diseases. The application of these nutrition in the food supplements of humans is one of the many aspects of biotechnology that has great impact on the society to fight against pandemics. By the year 2050, it is expected that more than 10 billion people will be living on this planet, and it is also believed that there may not be enough resources to feed the world population (UNFPA 1995) for healthy life. Hunger and malnutrition already claim 24,000 lives a day in developing countries such as Asia, Africa and Latin America (James 2003). Malnutrition, however, is not exclusive to developing nations. Many people of developed countries though considered well fed often suffer from improper nourishment resulting in poor mass immunity making the population vulnerable or susceptible to communicable diseases as evident during recent pandemic. Through nutrition biotechnology, scientists can enhance resistance to diseases and environmental stresses, afflicting the human society. Recent developments in the biotechnology of nutrition will allow the production of more nutritious, safer, tastier and healthier food. Advances in genetic engineering are revolutionizing the way we produce and consume food, and it is quite possible that in the next decade a large percentage of the food we eat will be bioengineered for safeguarding human health. Recent advances in Prebiotics, Probiotics and Nutraceuticals of biotechnology encompass continuously evolving methods or materials, for generating energy to non-toxic cleaning nutritional products. It is that innovation which reduces waste by changing patterns of production and consumption through DNA engineering. The book *Prebiotics, Probiotics and Nutraceuticals* contains 15 chapters and covers most of the core nutrition of both plant and animal origin, isolated and detected by our distinguished scientists. The objective of the book is to draw the attention of our future budding scientists, researchers and policy makers for the exploration and development of *Prebiotics, Probiotics and Nutraceuticals* in the frontier area for social welfare.

Balasore, Odisha, India — Kambaska Kumar Behera
Jaipur, Rajasthan, India — Renu Bist
Cuttack, Odisha, India — Sangita Mohanty
Balasore, Odisha, India — Manojit Bhattacharya

Acknowledgements

It is my privilege to express my deep sense of gratitude to Prof. (Dr.) Uttam Kumar Sahoo, Senior Professor & Dean, SES & NRM Mizoram (Central) University, Aizawl (Mizoram), for his kind consent to write the foreword for this volume.

I acknowledge the assistance, encouragement, excitement and efforts of the contributors in organizing and publishing this book. The excellent contents and uniqueness of each individual chapter will make this book as a textbook on the use of nutrition biotechnology and its frontier area for a moderately long term, which usually does not occur with books associated with this genre. I thank all the contributors for their understanding and patience. There are several people who deserve special thanks, but few of them are my beloved co-editor Dr. Manojit Bhattacharya, Dr. Renu Bist and Dr. Sangita Mohanty for their unconditional support and cooperation in making the project successful and fruitful.

Words are not enough to thank all my friends and colleagues who also inspired a lot to complete this work.

Last but not least, I would like to assert my intricate appreciation of gratitude and best regards to my loving family members for their incessant support and cooperation.

I acknowledge Mr. Gaurav Singh and Ms. Raman Shukla from Springer Nature, for their constant support and cooperation for publication of this volume.

Contents

1 Potentiality of Probiotics in Inactivation of Tetrodotoxin 1
Rudra Prasad Nath and Jayanta Kumar Kundu

2 Therapeutic Strategy for the Deterrence of COVID-19 with Relevance to Probiotics 13
Shubhita Mathur and Renu Bist

3 Plant-Based Bioactive Compounds in Cancer Therapeutics 29
Neha Jain, Pallavi Kaushik, Aparna Pareek, Hemant Pareek, and Mukesh Kumar Sharma

4 Nutraceuticals as Therapeutic Agents for Prevention and Treatment of Diseases 45
Harshita Sachdeva, Sarita Khaturia, and Mamta Chahar

5 Probiotics as Efficacious Therapeutic Option for Treating Gut-Related Diseases: Molecular and Immunobiological Perspectives 69
Nabarun Chandra Das, Ritwik Patra, Atrayee Dey, and Suprabhat Mukherjee

6 The Progressive Development of Probiotics, Prebiotics, and Synbiotics Research and Its Multipurpose Use in the Ornamental Fishery 95
Manab Kumar Saha, Manojit Bhattacharya, and Bidhan Chandra Patra

7 The Commercial Perspective of Probiotics and Bioremediating Components in Aquaculture Pond Management: A Case Study . . . 113
Prasenjit Barman, Kalyanbrata Pal, Suman Kumar Halder, and Partha Bandyopadhyay

8 Prebiotics as Promising Therapeutics for Treating Gut-Related Disorders: Biochemical and Molecular Perspectives 133
Ritwik Patra, Saubhik Mitra, Nabarun Chandra Das, and Suprabhat Mukherjee

9 Prebiotics and Probiotics as Functional Foods: Prospect and Promises in Metabolic Diseases 155
Tanuka Das, Tandrani Das, and Goutam Ghosh

10 Implications of Probiotics and Prebiotics on Immune Functions . . . 187
Kavita Pandey and Anam Modi

11 Recent Trends in Natural Medicines and Nutraceuticals Research 203
Ajaya Kumar Rout, Sujata Dey, Biswanath Patra, Manojit Bhattacharya, Asim Kumar Jana, Bhaskar Behera, and Bijay Kumar Behera

12 Nutraceuticals: An Alternative of Medicine 213
Rahul Kumar, Sourav Das, Santosh Kumar Sethi, Pankaj Kumar Parhi, and Birendra Kumar Bindhani

13 Socioeconomic Study of Prospective of Probiotics, Prebiotics, and Synbiotics for Sustainable Development of Aquaculture in Indian Sundarbans 253
Swagat Ghosh, Manojit Bhattacharya, and F. H. Rahaman

14 *Drosophila melanogaster* as an In Vivo Model for the Investigation of Host-Microbiota Interaction 275
Swetapadma Sahu, Pragalbha Jaysingh, and Monalisa Mishra

15 Prospectives of Prebiotics, Probiotics, and Synbiotics for Sustainable Development of Aquaculture 301
P. Kaushik, R. Khandelwal, N. Jain, S. Keelka, and P. Jain

About the Editors

Kambaska Kumar Behera, Ph.D. & Postdoc is a distinguished academician and researcher, published more than 100 research papers and six books of international and national repute. Born in Odisha, Dr. Behera has studied in Utkal University for his M.Phil. and Ph.D. degrees and owned the distinction of DBT-Postdoctoral Fellowship. He has worked on different research capacities in various national research institutes of India viz. CSIR, ICAR and NIT. He presently works as ASSISTANT PROFESSOR in the P.G. Department of Botany at Fakir Mohan University, Balasore, Odisha.

Renu Bist, Ph.D. currently serves as an **Associate Professor** in the Department of Zoology at the University of Rajasthan, Jaipur, India. As an academician and researcher, she supervised eight Ph.D. students and has 45 publications in journals of high repute. She is the author of two books of international and national repute. She is born in Udaipur, Rajasthan, and completed her M.Sc. and Ph.D. degrees in Mohanlal Sukhadia University, Udaipur. She has almost 15 years of teaching and research experience.

Sangita Mohanty, Ph.D. is an IARI Alumni and S. P. Raychowdhury Gold medallist, presently working as Senior Scientist, Crop Production Division, ICAR-**National Rice Research Institute**, Bidyadharpur, Cuttack, Odisha, India. She has more than 15 years of research experience in both international and national research organizations. She obtained her master's and doctoral degrees in soil science and agricultural chemistry from Indian Agricultural Research Institute, New Delhi. She has published more than 50 research articles in high-impact journals, book chapters and books of national and international repute. She has been deputed as visiting researcher to the University of Aberdeen, Scotland, UK, with NEWS India-UK Senior fellowship. She has handled more than ten research projects as lead and co-investigator and guided several Master science students in his research capacity in the field of soil nutrient management.

Manojit Bhattacharya, Ph.D. & Postdoc successfully completed his doctoral degree (Zoology) at Vidyasagar University, West Bengal, India. He published 60 research articles in peer-reviewed, high-impact journals and two books (as co-author). He has more than 8 years of operational experience in freshwater resources mapping, fish genomics and computational biological platforms. Currently, he serves as an Assistant Professor of Zoology at Fakhir Mohan University, Balasore, Odisha. Dr. Bhattacharya has wide expertise in different aspects of advanced molecular biology and bioinformatics.

Potentiality of Probiotics in Inactivation of Tetrodotoxin

1

Rudra Prasad Nath and Jayanta Kumar Kundu

Abstract

Tetrodotoxin (TTX) is a powerful neurotoxin principally spotted in the liver, skin and gonads of puffer fishes. TTX is also reported from different other organisms like goby, newts, frogs, blue-ringed octopus, gastropods, starfishes and xanthid crabs. TTX is more toxic than that of potassium cyanide (KCN), and the human nervous system will suffer badly when exposed to TTX. Mild exposure to TTX may lead to different problems like headache, lack of sensation of the lips, etc. Intoxication of TTX influences vomiting tendency, dizziness, lack of sensation, itching, increased heart rate, lowered blood pressure and paralysis of the skeletal muscles and diaphragm and at last can cause demise by arrestation of breathing. Toxicity of TTX is fairly widespread among Asian countries like Japan, China, Thailand, India, Bangladesh, etc. TTX can never be destroyed after cooking or refrigerating or by digestive juices inside the alimentary canal because it is thermostable and acid-stable. TTX is an effective neurotoxin which can particularly obstruct voltage-gated Na^+ channels present at the outer layer of the nerve membrane. So, transport of sodium ions through the cell membrane is blocked, and progression of nerve impulse is halted. Toxicity of tetrodotoxin (TTX) can be neutralised by some probiotics like exopolysaccharide (EPS) extract of a few lactic acid-producing bacteria. TTX detoxification ability of EPS of *Lactobacillus rhamnosus* PN04, *Lactobacillus plantarum* PN05 and *Leuconostoc mesenteroides* N3 was proven by different experiments. The entire assessment proved the significance of EPS of bacteria in the neutralisation of TTX. Exopolysaccharide (EPS) extracts from *Lactobacillus rhamnosus* PN04, *Lactobacillus plantarum* PN05 and *Leuconostoc mesenteroides* N3 were properly examined. Different types of investigations like high-performance liquid

R. P. Nath (✉) · J. K. Kundu
Department of Zoology, Vidyasagar University, Midnapore, West Bengal, India

K. K. Behera et al. (eds.), *Prebiotics, Probiotics and Nutraceuticals*,
https://doi.org/10.1007/978-981-16-8990-1_1

chromatography (HPLC) and thin-layer chromatography (TLC) were done to confirm the presence of glucose in most of the EPS of these bacteria.

Keywords

Tetrodotoxin (TTX) · Probiotics · Exopolysaccharide (EPS) · Puffer fish

1.1 Introduction

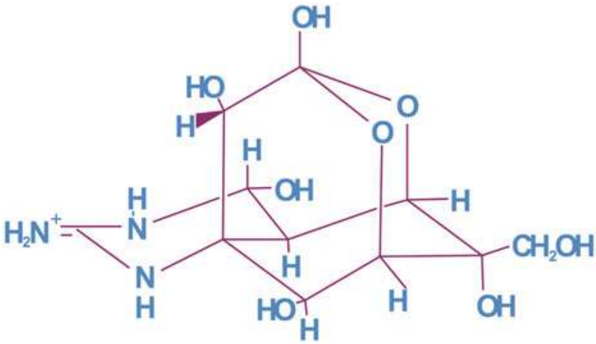
Tetrodotoxin (TTX) is especially an effective toxin present in tissues like the liver, skin, ovaries and testes of puffer fishes (Chau et al. 2011). Tetrodotoxin is named so because of its chief occurrence in puffer fishes of order Tetraodontiformes (Goto et al. 1965). TTX is not only reported from puffer fishes but also found in goby (Noguchi and Hashimoto 1973), California newts (Bucciarelli et al. 2014), frogs (Noguchi and Arakawa 2008), xanthid crabs (Noguchi et al. 1986), gastropod molluscs (Yang et al. 1995), blue-ringed octopus (Yotsu-Yamashita et al. 2007) and starfishes (Noguchi and Arakawa 2008). There is no such phylogenetic alliance between these TTX-containing animals. TTX is accumulated in different tissues of different organisms (Table 1.1) (Noguchi and Arakawa 2008).

TTX is predominantly an effective neurotoxin which can particularly barricade voltage-gated Na^+ channels present at the outer layer of the nerve membrane. So, transport of sodium ions through the cell membrane is blocked, and progression of nerve impulse is halted (Narahashi 2001; Benzer 2007). There was a controversy about the endogenous or exogenous property of TTX. Endogenous means that TTX is made up by puffer fish by its own, whereas exogenous is where TTX is isolated from the exterior and accumulated in puffer fish (Noguchi and Arakawa 2008). Several experiments recommended that puffer fish toxin is exogenous. The manufacture of TTX is done principally by marine bacteria and diverse parasitic or symbiotic bacteria which can be directly gathered inside the different tissues of

Table 1.1 Different TTX-containing animals (source: Noguchi and Arakawa 2008)

Animals	Toxic tissues
Arthropoda Xanthid crab (Family: Xanthidae)	Whole body
Mollusca Blue-ringed octopus (*Octopus maculosus*) Gastropoda (*Babylonia japonica*)	Posterior salivary gland, digestive gland
Echinodermata Starfish (Genus: *Astropecten*)	Whole body
Fish Puffer fish (Family: Tetraodontidae) Goby (*Gobius criniger*)	Liver, skin, gonads, etc. Viscera, skin, muscle, etc.
Amphibia California newt (Genus: *Taricha*) Frog (Genus: *Atelopus*)	Viscera, skin, muscle, egg, etc. Skin, ovary, egg, etc.

Fig. 1.1 Structure of TTX. (Source: Arakawa et al. 2010)



puffer fishes like the liver, skin, gonad, etc. (Noguchi and Arakawa 2008). Tetrodotoxin is basically a metabolic product of the host. TTX can be manufactured by different symbiotic bacteria, viz., *Vibrio alginolyticus*, *Pseudoalteromonas tetrodonis*, *Alteromonas tetraodonis*, *Shewanella algae*, *Shewanella putrefaciens*, etc. (Matsui et al. 1990; Yasumoto et al. 1986). TTX can be stored inside the puffer body via bioaccumulation process, and that is why toxicity of puffer fishes may fluctuate from season to season. Different tissues of puffer fishes are differentially toxic due to differential storage by bioaccumulation. These symbiotic bacteria survive in the intestinal lining of the puffer fishes (Noguchi et al. 1986a, b).

Many studies exposed that TTX is much more toxic than that of potassium cyanide (KCN) (Kaplan 2006) and the human nervous system suffered badly due to exposure to TTX (Tambyah et al. 1994). Mild introduction to TTX may lead to different problems like headache, lack of sensation of the lips, etc. Intoxication of TTX influences vomiting tendency, dizziness, lack of sensation, itching, increased heart rate, lowered blood pressure and paralysis of the skeletal muscles and diaphragm and at last can cause demise by arrestation of breathing (Cheng et al. 1968). Toxicity by TTX is fairly widespread among Asian countries like Japan, China, Thailand, India, Bangladesh, etc. (Noguchi and Ebesu 2001). TTX can never be destroyed after cooking or refrigerating or by digestive juices inside the alimentary canal because TTX is thermostable and acid-stable (Kuromi et al. 1979). There is no readily available antidote till date to overcome tetrodotoxin poisoning, but instant supportive management and watchful administration of atropine or neostigmine can restrain lethality (Xu et al. 2005).

6-Hydroxyl (-OH) groups and a guanidinium group which has positive charge are found in molecular structure of tetrodotoxin ($C_{11}H_{17}O_8N_3$) (Fig. 1.1) with a molecular weight of 319 Da (Hwang et al. 2007). Tetrodotoxin is not an alkaloid, a carbohydrate, or a steroid, and it is not like any conventional amino acid. From the seventeenth century, cases of puffer fish poisoning have been recorded (Clark et al. 1999) and are still a biggest threat although cases are declining (Arakawa et al. 2010; Kungsuwan 1993). Majority of puffer fishes have elevated level of TTX accumulation in tissues like the testis, liver, ovary, skin, etc. (Hwang and Noguchi 2007; Monrat et al. 2011; Anraku et al. 2013).

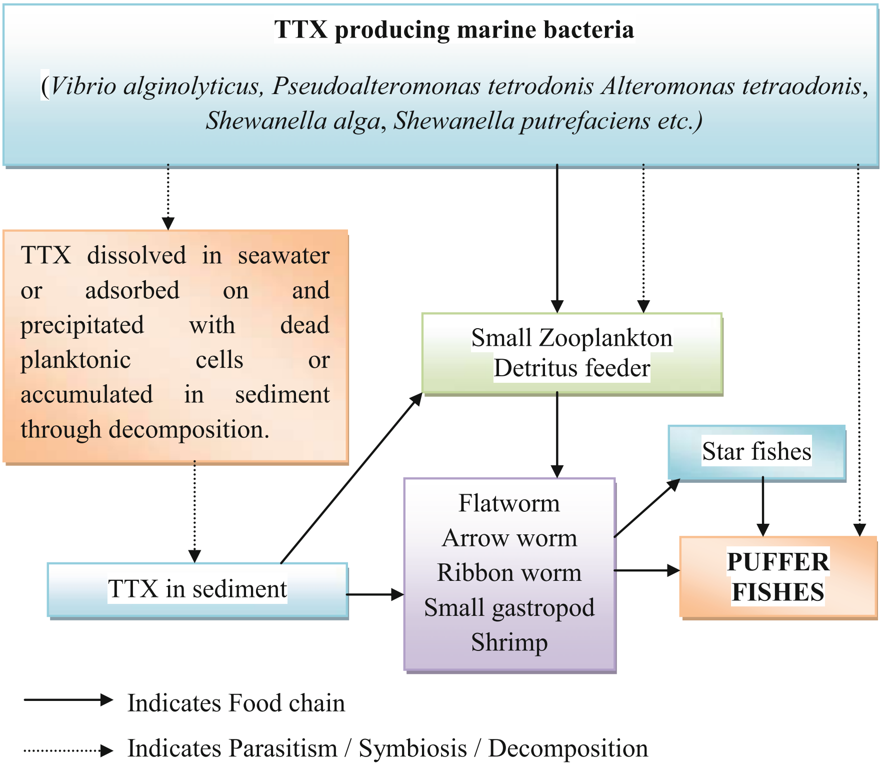


Fig. 1.2 Mechanism of TTX accumulation in puffer fishes (source: Noguchi and Arakawa 2008)

There are two ways by which TTX can be accumulated inside the body of puffer fishes. *In the first way*, TTX may be dissolved in saltwater or precipitated with deceased planktons or accumulated inside sediment in the course of breakdown and decomposition. Through the food chain, the primary consumers such as zooplankton, detritus feeder, flatworm, arrow worm, ribbon worm, small gastropod and shrimp will feed on dead planktonic cell on the sediment that consists of TTX followed by the secondary consumers like puffer fish, large gastropod, etc. feed on the primary consumers. As a consequence, the secondary consumers acquire TTX in their body. *The second way* is the TTX-producing marine bacteria like *Vibrio alginolyticus*, *Pseudoalteromonas tetrodonis*, *Alteromonas tetraodonis*, *Shewanella algae*, *Shewanella putrefaciens*, etc. which perform as parasite or build symbiotic relationship with zooplanktons and detritus feeder (Noguchi and Arakawa 2008). Through the food chain, the entire consumers will get the TTX. The illustration is shown in Fig. 1.2 (Noguchi and Arakawa 2008).

Several TTX derivatives have been isolated from puffer fishes, newts, frogs etc. till date. (Yotsu-Yamashita et al. 2007). These organic compounds are reported to possess biological activities like antitumor, antimicrobial, analgesic, etc. (Rajamanikandan et al. 2011; Narahashi 2001). TTX is applied as a potential pain relief, and the analgesic activity of TTX is studied to reduce extreme cancer pain, anxiety and drug abuse (Hagen et al. 2008; Joshi et al. 2006; Marcil et al. 2006; Shi et al. 2009). TTX is largely an efficient neurotoxin which can particularly blockade voltage-gated Na^+ channels present at the outer layer of the nerve membrane. So, transport of sodium ions through the cell membrane is blocked, and progression of nerve impulse is halted. This action takes place in the peripheral motor, sensory and autonomic nerves (Shi et al. 2009). The function of electrical impulses is to transmit information from the brain to different parts of the body. These signals work from dendrite to axon inside the nerve cell. The signals engage in the flow of K^+ ions and Na^+ ions via axon, along the plasma membrane of the nerve. Nerves require K^+ ions and Na^+ ions together to create specific electrical communication called as action potential. K^+ ions and Na^+ ions are travelling interior and exterior of the plasma membrane causing changes and carrying electrical message, but tetrodotoxin can be coupled with sodium ion, stopping its movement. Therefore, "message-sending" method will be deactivated, and the impulse certainly not reaches to its destination (Fig. 1.3) (Shi et al. 2009).

α-Subunits of Na^+ channel which is voltage-gated are made up with four similar domains like DI, DII, DIII and DIV. Every domain has six α-helical sections (1–6). Section 4 is brown in colour and denoted as voltage sensors. Locations of phosphorylation via protein kinase C (PKC) are denoted by brown-coloured boxes, and protein kinase A (PKA) is symbolised by yellow-coloured circles. Fast inactivation gate is positioned at intracellular loop connecting DIII and DIV and is denoted as H in green oval configuration. Helixes 1, 2 and 3 are fluorescent green in colour in this pictorial depiction. In between blue-coloured fifth helix and sixth helix, specific loops known as P-loops are positioned. The inner ring is represented by pink colour, and the outer ring is coloured by green in the picture. Mechanism of action of TTX is accompanied by amino acids between the pore of the outer ring and inner ring (Fig. 1.4) (Nieto et al. 2012).

Till date no specific antidote has been developed to prevent tetrodotoxin poisoning. Certain effective method is developed to curtail those hazards allied with TTX intoxication. In different researches, the roles of many probiotics are studied to overcome TTX toxicity. Lactic acid bacteria (LAB) are found to be the most successful to conquer TTX intoxication in several cases. Probiotics are actually live, good, helpful bacteria found in certain foods or supplements which keep the gut healthy. Probiotics can offer plentiful health benefits. Different foods and food supplements which are treated as probiotics are considered to be secure for many people; nevertheless, individuals having low immunity or past illness record should avoid the use of probiotics. In a few incidents, placid side effects like trouble of the stomach, diarrhoea, gastritis and allergic reactions may happen (Doron and Snydman 2015; Singhi and Kumar 2016; Durchschein et al. 2016).

Studies proved that exopolysaccharide (EPS) was one of the main compounds which bacteria produce to confer their resistance to harsh conditions or toxic environment. Research also exposed the piece of evidence that TTX was isolated from toxic tissues of puffer fishes by the process of conventional fermentation of Japanese. LAB has considerable the role in this procedure. Lactic acid bacteria may

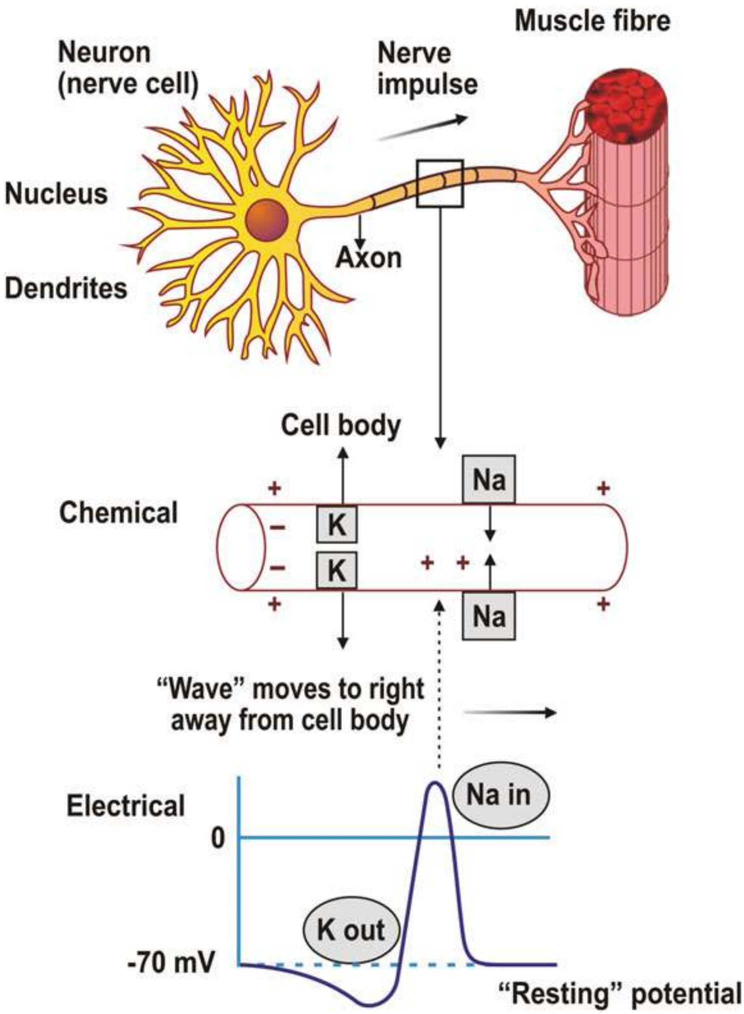


Fig. 1.3 Mechanism of generating action potential due to action of tetrodotoxin (source: Shi et al. 2009)

be of two types, i.e. homopolysaccharide bacteria and heteropolysaccharide bacteria, to keep them from adverse culture environment like desiccation, osmotic pressure, predation by protozoans, phagocytosis, antibiotics or toxic compounds and phage attack (Patel and Prajapat 2013; Rodriguez et al. 2003). There are quite a lot of heteropolysaccharides categorised in accordance with their structure, molecular weight, composition, function, etc. Production of exopolysaccharide from LAB is robustly subjective to culture setup (Iliev et al. 2006). Chronic gastritis can be checked by the exopolysaccharide made from *Streptococcus thermophilus* CRL 1190 (Rodriguez et al. 2009). Potential antioxidant properties were seen in the

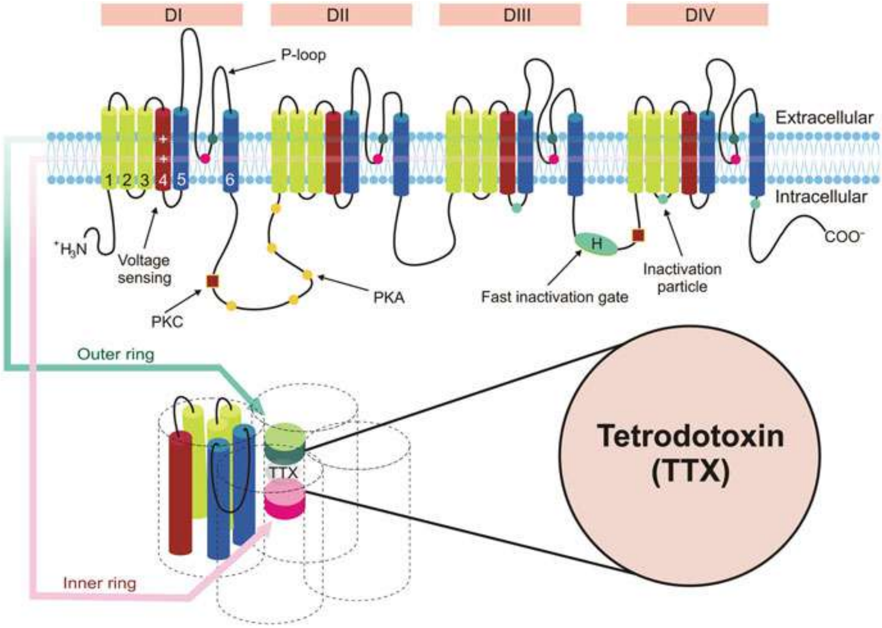


Fig. 1.4 Pictorial representation of interaction of α-subunit of Na^+ channel and coupling site of TTX (Nieto et al. 2012)

exopolysaccharide made up from two *Lactobacillus* species like *Lactobacillus plantarum* NTU 102 and *Lactobacillus paracasei paracasei* NTU 101 (Liu et al. 2011). TTX detoxification ability of EPS extracted from *Lactobacillus rhamnosus* PN04, *Lactobacillus plantarum* PN05 and *Leuconostoc mesenteroides* N3 is proved. The entire assessment proves the significance of EPS of bacteria in neutralisation of TTX. EPS yielded from the samples of *Lactobacillus rhamnosus* PN04, *Lactobacillus plantarum* PN05 and *Leuconostoc mesenteroides* N3 is experimented (Tu et al. 2014).

Detoxification properties of different strains of *Lactobacillus* sp. bacteria including *Lactobacillus plantarum* and *Lactobacillus rhamnosus* are determined in the direction of mycotoxins like deoxynivalenol, zearalenone, aflatoxin B1, fumonisins, etc. which are present in various contaminations of food (Chlebicz and Śliżewska 2020).

1.2 Results and Discussion

TTX detoxification abilities of different lactic acid bacteria, viz., *Lactobacillus rhamnosus* PN04, *Lactobacillus plantarum* PN05 and *Leuconostoc mesenteroides* N3, were tested in different studies. EPS is being produced by several bacteria in response to tough situation and caries out a variety of activities (Hall-Stoodley et al.

2004). EPS can combine directly to antibiotic and decrease the level of toxicity or put off active sites for binding of antibiotics. Different methods are applied like high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC). The investigation confirmed the presence of glucose in the majority of EPS of *Lactobacillus rhamnosus* PN04, *Lactobacillus plantarum* PN05 and *Leuconostoc mesenteroides* N3 (Tu et al. 2014). *Lactobacillus rhamnosus* PN04, *Lactobacillus plantarum* PN05 and *Leuconostoc mesenteroides* N3 have equivalent output of exopolysaccharide but, their water solubility is varied mostly due to disparity of functional groups, directing to the dissimilar relation with different atoms as well as water (Tu et al. 2018).

Exopolysaccharide derived from *Lactobacillus rhamnosus* PN04 and *Leuconostoc mesenteroides* N3 has specific ability of detoxification of TTX when amalgamated with cuprous oxide; on the other hand, the EPS of *Lactobacillus plantarum* PN05 without help of cuprous oxide individually can counteract TTX. Dissimilar molecular configuration of exopolysaccharide made up from these three *Lactobacillus* strains may result to disparity (Tu et al. 2018).

Different observations revealed that the exopolysaccharide acquired from *Lactobacillus plantarum* PN05 could be applied alone to avoid TTX contamination. On the other hand, the exopolysaccharide yielded from *Lactobacillus rhamnosus* PN04 and *Leuconostoc mesenteroides* N3 demonstrated TTX neutralisation capability while they are coupled to copper ion. Exopolysaccharide has long sequence polymer, but TTX encloses quite a lot of strong functional residues. Exopolysaccharide configuration of both *Lactobacillus rhamnosus* PN04 and *Leuconostoc mesenteroides* N3 in existence of copper ion includes different functional groups like N–H and O–H that can react with copper and form a multifaceted configuration which is capable of binding to TTX through several hydrogen (H) bonds (Fig. 1.5), and then TTX becomes unable to develop toxicity anymore. In accordance with FTIR data, the exopolysaccharide derived from *Lactobacillus plantarum* PN05 is found to be methylated. Electrical density of oxygen molecule turns into more negative by this methyl group which subsequently permits the oxygen molecule to bind with the hydrogen molecule present within hydroxyl (–OH) groups of tetrodotoxin (Fig. 1.6). This type of communications may take place more rapidly than the dealing of copper ion with nitrogen molecule of tetrodotoxin directing to inactivation of tetrodotoxin in the absence of copper ion (Tu et al. 2018).

1.3 Conclusion

From different studies, it is clear that exopolysaccharide derived from *Lactobacillus rhamnosus* PN04 and *Leuconostoc mesenteroides* N3 when complexed to cuprous oxide assists fairly to detoxify tetrodotoxin which is particularly observed on mouse model. Cuprous oxide alone is referred to as a toxic compound; so the mixture of cuprous oxide and exopolysaccharide obtained from *Lactobacillus rhamnosus* PN04 and *Leuconostoc mesenteroides* N3 should be considered as toxic as well. On the other hand, exopolysaccharide yielded from *Lactobacillus plantarum* PN05 can

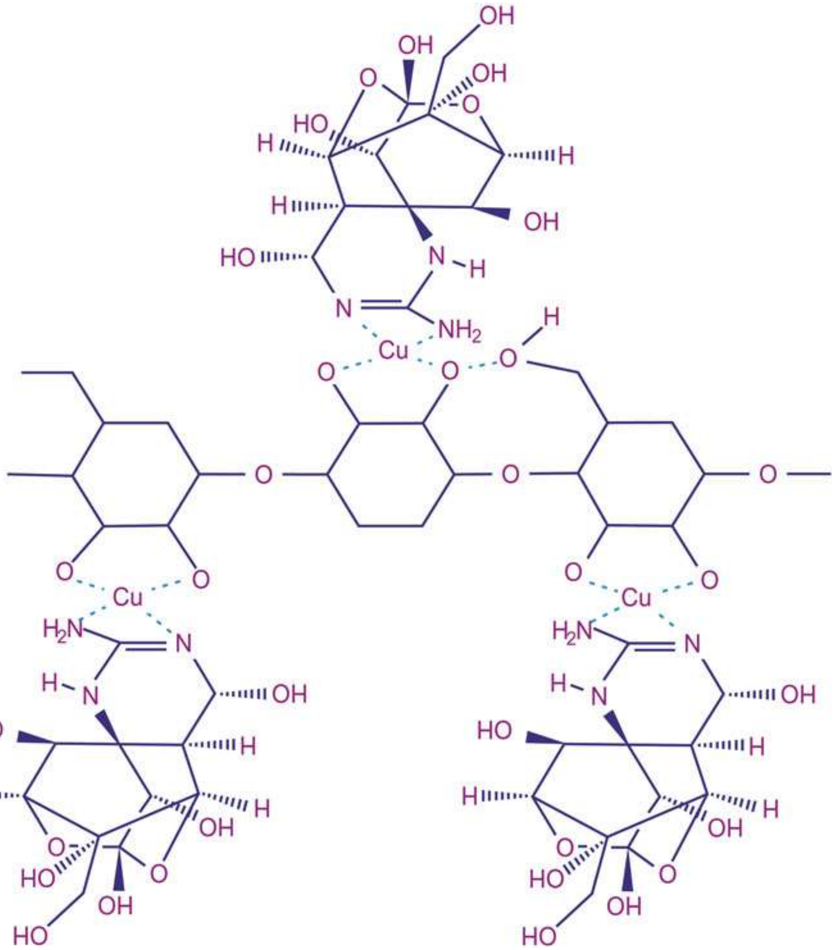


Fig. 1.5 This model depicts relation of TTX and EPS from *Lactobacillus rhamnosus* PN04 and *Leuconostoc mesenteroides* N3 via copper bridges (Tu et al. 2018)

detoxify tetrodotoxin by its own in experimental mice model. Consequently, more studies possibly will disclose the promising ways of neutralisation of tetrodotoxin in human also.

Acknowledgment The authors wish to record their thanks to scientists of MARC Digha, Regional Centre of ZSI, Department of Zoology, Vidyasagar University, for giving valuable suggestions during this study. The authors are also grateful to Mr. Soumitra Samanta for his constant support during this survey.

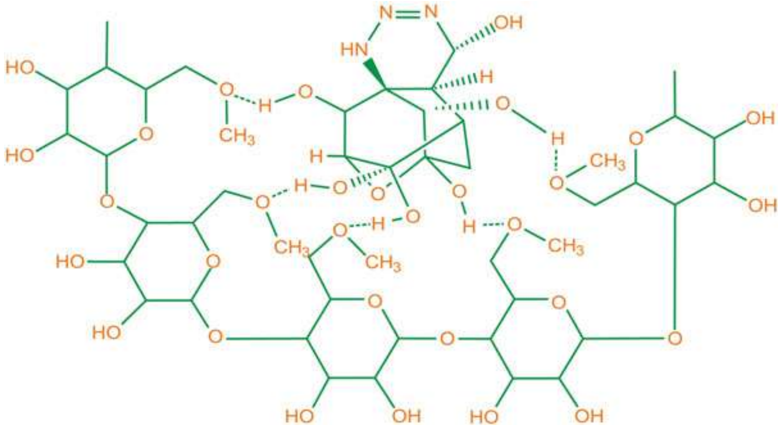


Fig. 1.6 The model shows interaction between TTX and EPS derived from *Lactobacillus plantarum* PN05 (Tu et al. 2018)

References

Anraku K, Nonaka K, Yamaga T, Yamamoto T, Shi MC, Wakita M, Hamamoto A, Akaike N (2013) Removal of toxin (Tetrodotoxin) from puffer ovary by traditional fermentation. Toxins 5:193–202

Arakawa O, Hwang D, Taniyama S, Takatani T (2010) Toxins of puffer fish that cause human intoxications. In: Ishimatsue A, Lie HJ (eds) Coastal environmental and ecosystem issues of the east China sea. Nagasaki University, Nagasaki, pp 227–244

Benzer TI (2007) Toxicity. Tetrodotoxin. http://emedicine.medscape.com. Accessed: March 2015

Bucciarelli GM, Li A, Zimmer RK, Kats LB, Green DB (2014) Quantifying tetrodotoxin levels in the California newt using a non-destructive sampling method. Toxicon 80:87–93

Chau R, Kalaitzis JA, Wood SA, Neilan BA (2011) On the origins and biosynthesis of tetrodotoxin. Aquat Toxicol 104:61–72

Cheng KK, Ling YL, Wang JCC (1968) The failure of respiration in death by tetrodotoxin poisoning. Exp Physiol 53:119–128

Chlebicz A, Śliżewska K (2020) In vitro detoxification of aflatoxin B1, deoxynivalenol, fumonisins, T-2 toxin and zearalenone by probiotic bacteria from genus Lactobacillus and Saccharomyces cerevisiae yeast. Probiot Antimicrob Proteins 12:289–301

Clark RF, Williams SR, Nordt SP, Manoguerra AS (1999) A review of selected seafood poisonings. Undersea Hyperb Med 26:175–184

Doron S, Snydman DR (2015) Risk and safety of probiotics. Clin Infect Dis 60(Suppl 2):S129–S134

Durchschein F, Petritsch W, Hammer HF (2016) Diet therapy for inflammatory bowel diseases: the established and the new. World J Gastroenterol 22(7):2179–2194

Goto T, Kishi Y, Takahashi S, Hirata Y (1965) Tetrodotoxin. Tetrahedron 21:2059–2088

Hagen NA, Du Souich P, Lapointe B, Ong-Lam M, Dubuc B, Walde D, Love R, Ngoc AH (2008) Tetrodotoxin for moderate to severe cancer pain: a randomized, double blind, parallel design multicenter study. J Pain Symptom Manag 35:420–429

Hall-Stoodley L, Costerton JW, Stoodley P (2004) Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol 2:95–108

Hwang DF, Noguchi T (2007) Tetrodotoxin poisoning. Adv Food Nutr Res 52:141–236

Hwang PA, Tsai YH, Lin SJ, Hwang DF (2007) The gastropods possessing TTX and/or PSP. Food Rev Int 23:321–340

Iliev I, Ivanova I, Ignatova C (2006) Glucansucrases from lactic acid bacteria (Lab). Biotechnol Equip 20:15–20

Joshi SK, Mikusa JP, Hernandez G, Baker S, Shieh CC, Neelands T, Zhang XF, Niforatos W, Kage K, Han P (2006) Involvement of the TTX-resistant sodium channel Nav 1.8 in inflammatory and neuropathic, but not post-operative, pain states. Pain 123:75–82

Kaplan EH (2006) Sensuous seas: tales of a marine biologist. Princeton University Press, Princeton

Kungsuwan A (1993) Survey on poisonous puffer fish in Andaman Seas. Proceedings of the seminar on Fishes, Department of Fisheries, Sept 15–17, Bangkok, Thailand

Kuromi H, Gonoi T, Hasegawa S (1979) Partial purification and characterization of neurotrophic substance affecting tetrodotoxin sensitivity of organ-cultured mouse muscle. Brain Res 175: 109–118

Liu CF, Tseng KC, Chiang SS, Lee BH, Hsu WH, Pan TM (2011) Immunomodulatory and antioxidant potential of lactobacillus exopolysaccharides. J Sci Food Agric 91:2284–2291

Marcil J, Walczak JS, Guindon J, Ngoc AH, Lu S, Beaulieu P (2006) Antinociceptive effects of tetrodotoxin (TTX) in rodents. Br J Anaesth 96:761–768

Matsui T, Taketsugu S, Sato H, Yamamori K, Kodama K, Ishi A, Hirose H, Shimizu C (1990) Toxification of cultured puffer fish by the administration of tetrodotoxin producing bacteria. Nippon Suisan Gakkaishi 56(4):705

Monrat C, Nitat S, Srimanote P, Indrawattana N, Thanongsaksrikul J, Chongsa-Nguan M, Kurazono H, Chaicumpa W (2011) Toxic marine puffer fish in Thailand seas and tetrodotoxin they contained. Toxins 3(10):1249–1262

Narahashi T (2001) Pharmacology of tetrodotoxin. J Toxicol 20:67–84

Nieto FR, Cobos EJ, Tejada MÁ, Fernández CS, González-Cano R, Cendán CM (2012) Tetrodotoxin (TTX) as a therapeutic agent for pain. Mar Drugs 10:281–305

Noguchi T, Arakawa O (2008) Tetrodotoxin-distribution and accumulation in aquatic organisms, and cases of human intoxication. Mar Drugs 6:220–242

Noguchi T, Ebesu JSM (2001) Puffer poisoning: epidemiology and treatment. Toxin Rev 20:1–10

Noguchi T, Hashimoto Y (1973) Isolation of tetrodotoxin from a goby Gobius criniger. Toxicon 11: 305–307

Noguchi T, Jeon JK, Arakawa O, Sugita H, Deguchi Y, Shida Y, Hashimoto K (1986a) Occurrence of tetrodotoxin in *Vibrio* sp. isolated from intestines of xanthid crab, *Atergatis floridus*. J Biochem 99:311–314

Noguchi T, Arakawa O, Daigo K, Hashimoto K (1986b) Local differences in toxin composition of a xanthid crab Atergatis floridus inhabiting Ishigaki Island, Okinawa. Toxicon 24:705–711

Patel A, Prajapat JB (2013) Food and health applications of exopolysaccharides produced by lactic acid bacteria. Adv Dairy Res 1:1–8

Rajamanikandan S, Sindhu T, Durgapriya D, Anitha JR, Akila S, Gopalakrishnan VK (2011) Molecular docking and QSAR studies on bioactive compounds isolated from Marine organisms into the MUCI Onco protein. Int J Pharm Sci 3:168–172

Rodriguez JM, Martinez MI, Horn N, Dodd HM (2003) Heterologous production of bacteriocins by lactic acid bacteria. Int J Food Microbiol 80:101–116

Rodriguez C, Medici M, Rodriguez AV, Mozzi F, Font de Valdez G (2009) Prevention of chronic gastritis by fermented milks made with exopolysaccharide-producing Streptococcus thermophilus strains. J Dairy Sci 92:2423–2434

Shi J, Liu TT, Wang X, Epstein DH, Zhao LY, Zhang XL, Lu L (2009) Tetrodotoxin reduces cue-induced drug craving and anxiety in abstinent heroin addicts. Pharmacol Biochem Behav *92*:603–607

Singhi SC, Kumar S (2016) Probiotics in critically ill children. F1000Res 5:407

Tambyah PA, Hui KP, Gopalakrishnakone P, Chin NK, Chan TB (1994) Central nervous system effects of tetrodotoxin poisoning. Lancet 343:538–539

Tu N, Tu Q, Tung H, Hieu D, Romero-Jovel S (2014) Detection of tetrodotoxin-producing Providencia rettgeri T892 in Lagocephalus puffer fish. World J Microbiol Biotechnol 30: 1829–1835

Tu NHK, Dat NV, Canh LV, Vinh DTT (2018) Detection of the potential inactivation of tetrodotoxin by lactic acid bacterial exopolysaccharide. Toxins 10:288

Xu QH, Wei CH, Huang K, Rong KT (2005) Toxin-neutralizing effect and activity-quality relationship for mice tetrodotoxin-specific polyclonal antibodies. Toxicology 206:439–448

Yang CC, Han KC, Lin TJ, Tsai WJ, Deng JF (1995) An outbreak of tetrodotoxin poisoning following gastropod mollusc consumption. Hum Exp Toxicol 14:446–450

Yasumoto T, Yasumura D, Yotsu M, Michishita T, Endo A, Kotaki Y (1986) Bacterial production of tetrodotoxin and anhydrotetrodotoxin. Agric Biol Chem 50(3):793–795

Yotsu-Yamashita M, Mebs D, Flachsenberger W (2007) Distribution of tetrodotoxin in the body of the blue-ringed octopus (*Hapalochlaena maculosa*). Toxicon 49:410–412

Therapeutic Strategy for the Deterrence of COVID-19 with Relevance to Probiotics

2

Shubhita Mathur and Renu Bist

Abstract

The epidemiology of novel corona virus disease (COVID-19) is attributed by cough, fever, fatigue, headache, sore throat, gastrointestinal disorders, and pneumonia. The frequency of COVID-19 infection is accelerating at great speed globally, and till date, there is no specific vaccine or drug available for prevention or cure of the disease. Therefore, certain measures are required to check the outbreak of this pandemic immediately. Current chapter concerns with the development of certain probiotics including a single or mixed culture of live microorganisms that could maintain the intestinal or lung microbiota of humans. Besides being beneficial bacteria, probiotics also possess antiviral activity. The stimulation of the immune system through probiotics is one of the approaches that have been emphasized for fighting against the viral infections. The immunomodulatory activities of probiotics include the enhancement of the phagocytic receptors like CR1, CR3, FccRI, and FcaR; induction of APC-derived pro- and anti-inflammatory cytokines such as IL-10, IL-12, IL-17, TNF-α, and IFN-α against foreign antigens; and an increase in the microbicidal function of neutrophils. The most common probiotics include *Lactobacillus*, *Bifidobacterium*, *Leuconostoc*, *Pediococcus*, and *Enterococcus*. *Lactobacillus* and *Bifidobacterium* are widely used in yogurts and other dairy products. Probiotics exert antiviral activity by production of antiviral in inhibitory metabolites.

S. Mathur
Jaipur National University, Jaipur, Rajasthan, India

R. Bist (✉)
Department of Zoology, University of Rajasthan, Jaipur, Rajasthan, India

K. K. Behera et al. (eds.), *Prebiotics, Probiotics and Nutraceuticals*,
https://doi.org/10.1007/978-981-16-8990-1_2

Keywords

Probiotics · COVID-19 · SARS-CoV-2 · Clinical trials · Hypoxemia · ARDS

2.1 Introduction

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 was first reported in Wuhan, China, in December 2019. Subsequently, the disease has spread worldwide. Coronaviruses (CoVs) are spherical or pleomorphic, enveloped, positive-stranded RNA viruses (+ssRNA) accompanied by long surface spikes. These crown-like viruses belong to the family *Coronaviridae* and order *Nidovirales*. The CoVs consist of four genera within the subfamily *Orthocoronavirinae*: *Alphacoronavirus* (alpha CoV), *Betacoronavirus* (beta CoV), *Deltacoronavirus* (delta CoV), and *Gammacoronavirus* (gamma CoV) (Yang and Leibowitz 2015; Li et al. 2020a, b). Among these, the alpha CoV and beta CoV have emerged as major human pathogens since they have the ability to cross animal-human barriers (Coleman and Frieman 2014; Zhu et al. 2020). Till date, seven groups of human coronaviruses (hCoVs) have been recognized that can infect humans. This includes the beta-genera CoVs such as severe acute respiratory syndrome (SARS)-CoV, Middle East respiratory syndrome (MERS)-CoV, hCoV-HKU1, and hCoV-OC43, the α-genera CoV such as hCoV-NL63, and the novel coronavirus that causes COVID-19 which is officially named as SARS-CoV-2 (Weiss 2020; Gorbalenya et al. 2020; Zhu et al. 2020). The ability of this virus to mutate and infect nonimmune populations has emerged as an ongoing global threat. Globally, as of 6 December 2020, there have been 65,870,030 confirmed cases of COVID-19, including 1,523,583 deaths, according to the World Health Organization (WHO) COVID-19 dashboard. There are no clinically approved medicines or vaccines which could provide therapy for COVID-19. In 2001, the definition of "probiotics" was reframed by the Food and Agriculture Organization of the United Nations (FAO) and the WHO as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill et al. 2014). Probiotics are used to impart useful functions to gut microbial communities by promoting microbial diversity within human microbiome. The inflammation of the gut and other disease phenotypes are averted as probiotics reinstate the composition of the gut microbiome. These live microorganisms are used to treat microbial deficiencies in the form of microbial supplementation. The representative symptoms of COVID-19 include fever, cough, myalgia, fatigue, and pneumonia (Guan et al. 2020; Huang et al. 2020). The less familiar symptoms include sputum production, headache, hemoptysis, and diarrhea (Huang et al. 2020). Few patients with COVID-19 exhibited intestinal microbial dysbiosis and decreased probiotics such as *Lactobacillus* and *Bifidobacterium*; hence, nutritional and gastrointestinal function must be evaluated for all patients. The application of prebiotics or probiotics can aid in the regulation of the balance of intestinal microbiota and, thus, reduces the risk of secondary infection.

2.2 Pathogenesis of COVID-19

The transmission of COVID-19 causing virus is predominantly via infective respiratory droplets. The mucosal surfaces of the host (eyes, nose, and mouth) get exposed to these droplets when an infected person is in close contact with someone who is actively coughing or sneezing (Li et al. 2020a, b). The incubation period of COVID-19, also known as "presymptomatic period," is 5–6 days but can be up to 14 days. COVID-19 patients at the time of admission in hospital exhibit a fever and dry cough, diarrhea, muscle and/or joint pain, nausea, headache/dizziness, struggle for breathing, and coughing up of blood (Tay et al. 2020).

Briefly, following three phases of COVID-19 correlates to different clinical stages of the disease (Wu and McGoogan 2020; Mason 2020).

Phase 1: Asymptomatic State (Mason 2020)

This phase comprises of initial 1–2 days of infection. During this phase, the inhaled virus SARS-CoV-2 replicates and fixes itself to epithelial cells in the nasal cavity. The main receptor and cofactor for SARS-CoV-2 entry in cells are angiotensin I-converting enzyme 2 (ACE-2) and transmembrane serine protease 2 (TMPRSS2) (Mason 2020; Hoffmann et al. 2020).

Phase 2: Respiratory Tract Response

A robust immune response initiates upon proliferation and migration of virus down the respiratory tract (Fig. 2.1) (Yang et al. 2020). However, expression of type I interferons (IFNs) by infected human monocyte-derived dendritic cells (DCs) and macrophages is inhibited by SARS-CoV (Cheung et al. 2005; Spiegel et al. 2005). The chemokine ligand CXCL10 is useful as disease marker in SARS (Tang et al. 2005; Rockx et al. 2009; Mason 2020). The blood of SARS-CoV-infected patients shows an increased expression of CXC chemokine ligand 10 (CXCL10) and C-C motif chemokine ligand 2 (CCL2) (Wong et al. 2004).

Phase 3: Respiratory Failure, "Happy" Hypoxemia, and Succession to ARDS

The low oxygenation index exhibited by SARS-CoV-2-infected patients leads to severe respiratory failure. As infection progresses, virus infects alveolar type II cells. Multifocal bilateral patchy shadows and/or ground glass opacities are observed in the chest computed tomography (CT) scans of infected patients (Mason 2020; Li et al. 2020a, b). A sensation of "uncomfortable, difficult, or labored" breathing is known as dyspnea. Several patients exhibit distinct arterial hypoxemia but lack relative signs of respiratory distress without the expression of dyspnea. This phenomenon in the infected patients is described as silent or "happy" hypoxemia (Tobin et al. 2020; Couzin-Frankel 2020). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-infected patients have characteristic viral pneumonia leading to acute respiratory distress syndrome (ARDS). Diffuse alveolar damage is caused by ARDS. During acute stage, alveoli exhibit hyaline membrane formation followed by interstitial widening, edema, and then fibroblast proliferation in the organizing stage. As disease progresses, COVID-19 ARDS patients exhibit fibrosis in the lungs (Gibson et al. 2020).

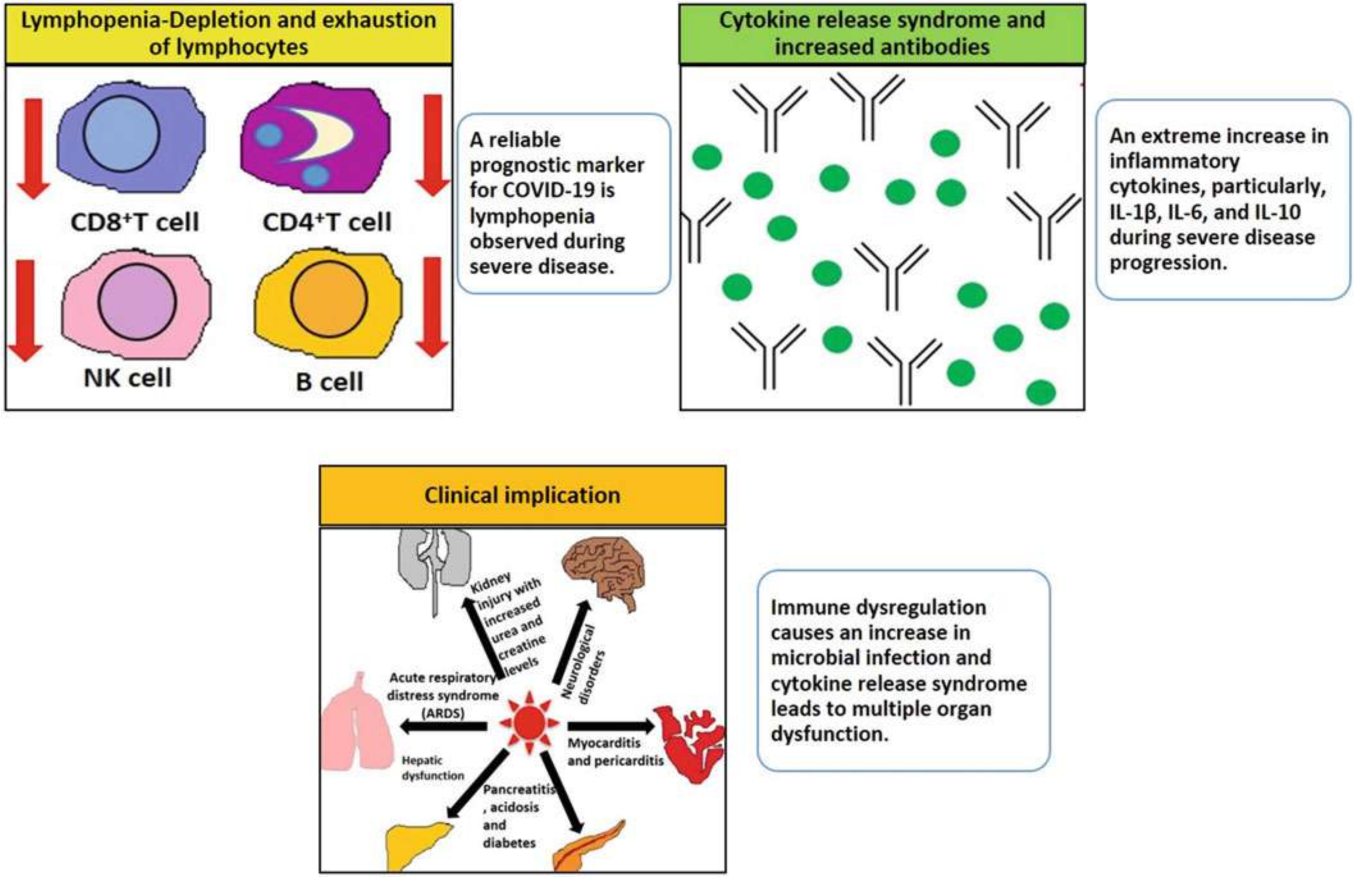


Fig. 2.1 Immunopathology and clinical implications of SARS-CoV-2 during disease progression

2.3 Clinical Data Supporting the Use of Probiotics to Prevent Viral Infections

NEC (necrotizing enterocolitis) is the most common acquired disease of the gastrointestinal tract in preterm infants. Probiotics are used for the prevention of NEC and late-onset sepsis and to decrease mortality in preterm infants. In a meta-analysis study, which includes very low birth weight (VLBW) preterm infants (weighing $<$1500 g), 30 randomized trials (RCTs) with more than 8000 preterm infants and 14 observational studies with more than 13,000 preterm infants, it is claimed that the use of probiotics is safe and feasible with a statistically significant reduction in NEC incidence, late-onset sepsis, and mortality. *L. acidophilus* in combination with *B. infantis* reduced NEC and mortality rate statistically significantly in comparison to the use of single strain such as *L. reuteri*, *B. breve*, or *S. boulardii* (Dermyshi et al. 2017). In developing countries like India, no efficient means for the prevention of sepsis is available. The Odisha State of India has the highest neonatal and infant mortality rates. A community-based, double-blind, placebo-controlled randomized trial in 149 arbitrarily selected villages in Odisha State; synbiotic (an amalgamation of probiotics with a prebiotic, the latter supplemented to stimulate growth and support colonization of the probiotic strain) was administered orally to the newborns for 7 days beginning on day 2–4 of life. The findings of the study suggested that a synbiotic containing *L. plantarum* ATCC-202195 could prevent neonatal sepsis in developing countries (Panigrahi et al. 2017). Most of the upper respiratory tract infections (RTIs) are of viral etiology. Probiotics diminutize RTIs and GI (gastrointestinal) symptoms in children, adults, and elderly. The outcome of probiotics varies on the quantity ingested and the pattern of consumption. *Lactobacillus rhamnosus* GG (LGG) moderately lowers the period of respiratory tract infections in comparison to placebo group (Laursen and Hojsak 2018). Mice infected with influenza virus were protected against influenza virus infection of the lower respiratory tract, augmented anti-influenza IgG production, and were precluded from death upon oral immunization with *Bifidobacterium breve* YIT4064 in comparison with mice immunized with influenza virus only (Yasui et al. 1999). A randomized, double-blind, placebo-controlled intervention study provides encouraging results of intake of *Lactobacillus gasseri* PA 16/8, *Bifidobacterium longum* SP 07/3, and *Bifidobacterium bifidum* MF 20/5 which lessened the intensity of common cold in healthy adults (de Vrese et al. 2005). In a placebo-controlled trial, 94 preterm infants with a birth weight more than 1500 g when administered orally with a prebiotic mixture consisting of galactooligosaccharide and polydextrose and a probiotic *Lactobacillus rhamnosus GG* exhibited significantly lower incidence of RTIs particularly rhinovirus infections compared with those receiving placebo (Luoto et al. 2014). In an open-label, parallel group trial, it was found that repeated consumption of fermented foods containing probiotics is useful for preventing influenza (Waki et al. 2014). In this study, 1089 school children exhibited reduced influenza infections when administered with a probiotic drink containing plant-derived lactic acid bacterium *Lactobacillus brevis* KB290 (Waki et al. 2014). Pandemic affects adults more than children. In elderly, immune capacity needs to be augmented as NK cell activity and acquired immunity are reduced by half as compared to middle-aged adults. The lowered immunity triggers influenza infection. The bactericidal activity of neutrophils builds up upon ingestion of bifidobacteria and lactic acid bacteria. NK cell and neutrophil bactericidal activities were significantly improved in 27 elderly subjects by consumption of *Bifidobacterium longum* BB536 (*B. longum BB536*), thus speculating that continuous ingestion of *B. longum BB536* diminishes the incidence of influenza and fever, possibly by reinforcing innate immunity (Namba et al. 2010). A meta-analysis that included 8 RCTs with 1083 participants demonstrates that people receiving probiotics experienced significantly reduced ventilator-associated pneumonia (VAP) in comparison to the control groups. All RCTs under investigation included probiotics such as *Lactobacillus casei rhamnosus*, *Lactobacillus plantarum*, Synbiotic 2000FORTE, *Ergyphilus*, and a combination of *Bifidobacterium longum* + *Lactobacillus bulgaricus* + *Streptococcus thermophilus* (Bo et al. 2014). Infant morbidity is mainly caused by respiratory infections. In a randomized, placebo-controlled, double-blind study, it was found that infant formula supplemented with probiotics *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb-12 decreases the possibility of primary acute otitis media (Rautava et al. 2008). Probiotics that exhibit gastrointestinal benefits do not necessarily contribute to reduce the risk of respiratory infection. For instance, though *Lactobacillus rhamnosus* GG and *Bifidobacterium animalis* ssp. impart intestinal benefits, however, they do not help to decrease the virus number in the nasopharynx.

2.4 Current Intervention Strategies for COVID-19

Presently, no particular antiviral therapeutic agents or vaccine for SARS-CoV-2 is available for protecting the infected patients or to safeguard health-care workers and others at elevated risk of infection. World Health Organization (2020) and its partners on 18 March 2020 launched one of the biggest international randomized trials known as Solidarity Trial for finding an effective treatment for COVID-19. The study registered approximately 12,000 patients in 500 hospital sites in over 30 countries. The aim of the Solidarity Trial is to assess the effect of drugs on COVID-19 patients to study the outcomes such as mortality, need for assisted ventilation, and duration of hospital stay (World Health Organization 2020; Kumar and Al Khodor 2020).

2.5 Probiotics for the Prevention of COVID-19

Probiotics exert anti-inflammatory, antiviral effects in vitro, in animal models and in humans. The human trials of probiotics may provide an ancillary evidence or hypothesis-driven methodology to explore the use of probiotics as adjunctive therapy in the prophylaxis and/or mitigation of COVID-19 symptoms.

Angiotensin-converting enzyme 2 (ACE2) belongs to the family of membrane-bound carboxydipeptidase (Kai and Kai 2020). ACE2 is expressed in vascular endothelia, renal and cardiovascular tissue, and epithelia of the small intestine and testes (Jia et al. 2005). ACE2 is extensively dispersed in the human body, including the heart, kidney, small intestine, and, to a lesser extent, the lung (Kai and Kai 2020). SARS-CoV-2 enters the host target cells via a functional receptor ACE2. The transmembrane spike (S) glycoprotein of the virus comprises of 1255 amino acids and facilitates entry of the virus into target cells by involving the ACE2 enzyme (Kai and Kai 2020). The S-protein is manufactured in the secretory pathway of infected cells. The binding of the SARS-CoV-S-protein to ACE2 is important for SARS-CoV infection. The S-protein trimers are fused with the viral envelope and the plasma membrane of the host cell. Cleavage by TMPRSS2 regulates the spread of virus infection within the host. The major viral target cells are type II pneumocytes. On these cells, transmembrane protease serine 2 (TMPRSS2) co-expresses itself with the SARS-CoV receptor ACE2 (Glowacka et al. 2011). ACE2 receptor is a homolog of the angiotensin I-converting enzyme (ACE) receptor which is a type I transmembrane aminopeptidase with high expression in the heart, lung tissue, endothelium, and kidney (Rico-Mesa et al. 2020). The expression of ACE2 is significantly amplified in patients administered with ACE inhibitors (Wan et al. 2020). Probiotic strains such as *Bifidobacteria (B.) bifidum* MF 20/5 produce peptides with ACE inhibitory activity through the proteolysis and fermentation of milk proteins (Gonzalez-Gonzalez et al. 2013). Proteolytic activity and ACE inhibition of *Lactobacillus* and *Bifidobacterium* strains were consistently amplified using enhanced fermentation substrates or prebiotics like inulin, mannitol, pectin, and fructo-oligosaccharides (Ettinger et al. 2014). However, the impending role of probiotic in regulating ACE2 level is to be analytically measured when they are proposed as an adjunctive therapeutic option (Fuglsang et al. 2003). High cholesterol levels are linked with additional lipid rafts which provide a docking site for ACE2 receptors for the S-protein of SARS-CoV-2. Thus, therapies that lower the level of cholesterol in decreasing viral infectivity are required (Radenkovic et al. 2020). *Bifidobacterium longum* 5022 (BL 5022), *L. acidophilus* CSCC 2404 (LA2404), *L. acidophilus* CSCC 2410 (LA 2410), and *Bifidobacterium bifidum* CSCC 5286 (BB 5286) exhibit higher cholesterol removal ability. Even nonviable probiotics may be used as cholesterol-reducing agents in the gastrointestinal system (Miremadi et al. 2014). Thus, probiotics may act as potential blocker to the ACE receptor of GI cells. COVID-19 patients who have low immunity demonstrate intestinal microbial dysbiosis identified by reduced number of probiotic species such as *Bifidobacterium* and *Lactobacillus*. Consequently, these patients need nutritional aid through probiotic supplementation in order to restore the intestinal flora (Xu et al. 2020). In spite of the lack of solid evidence supporting above treatments for COVID-19 infections, the above-discussed RCTs and observations for other virus infections support the administration of probiotics to COVID-19 patients. Moreover, the natural immunity of the patients can be boosted by using probiotics.

The following points need to be considered while using probiotics for protection against respiratory infections including COVID-19:

1. The selection of effective strains of probiotics is important because protective effects against influenza virus infection significantly vary among *Lactobacillus* strains. Furthermore, mice administered with intranasal administration of *Lactobacillus* exhibited higher survival rate as compared to mice receiving oral administration (Youn et al. 2012).
2. Adequate quantity of probiotics (>7 log CFU) is required to exhibit protective effect against COVID-19 and other respiratory tract infections (Olaimat et al. 2020).
3. Previous studies show that commercial probiotics do not survive food processing, storage, and passage through the upper GIT and, thus, exhibit 10^6-fold reduction in colony-forming units (CFU) within 5-min incubation in gastric fluids (Shori 2017). The efficiency of probiotics such as *Lactobacillus* and *Bifidobacterium* (Cook et al. 2012) and next-generation probiotic candidates such as *Akkermansia muciniphila* (Cani and de Vos 2017) and *Faecalibacterium prausnitzii* (Tochio et al. 2018; Lopez-Siles et al. 2017) for improving human health through nutritional interventions is restricted by severe conditions, such as bile salts, gastric acids, and oxygen, which will also limit their effectiveness for enhancing human health through nutritional interventions (Lopez-Siles et al. 2017). Subsequently, effective approach is required to ameliorate the stability of probiotics during their passage through the human gut (Yao et al. 2020). Thus, microencapsulation of probiotic cells into an encapsulating matrix or membrane that can protect the cells from bile salts, low pH, etc., which they encounter during gastrointestinal transit, is required (Olaimat et al. 2020; Muthukumarasamy et al. 2006) for protection against respiratory infections.

Microbiota status of host, duration of treatment, time of probiotic administration, requirement of booster dose, and adjunctive therapy are some of the factors that can be considered during clinical trials with probiotics for preventing diseases such as COVID-19 (Table 2.1).

2.6 Conclusion

As of yet, to the best of our knowledge, the use of probiotics for treatment or prevention of COVID-19 has not been reported by any study. However, one of the suitable applications of the probiotics can be found as an adjunctive therapy in clinical treatments. Benefits of probiotic administration in the background of COVID-19 infection are, primarily, due to their effects on innate and adaptive immunity. Previous studies/clinical trials may be employed to propose the following applications of probiotics in decreasing the gravity of SARS-CoV-2 infections:

1. Since SARS-CoV-2 bind ACE2 receptors for entry in the lungs and gut, probiotics may be utilized to regulate AEC2 which will help in lessening inflammations.
2. Probiotic therapy reduces both upper and lower respiratory infections.

Table 2.1 List of few trials registered for evaluation of the benefits of probiotic exposure in COVID-19-infected patients

Study title	Aim and study type	Intervention and probiotics	Primary outcome	Secondary outcome	References
Changes in viral load in patients with COVID-19 disease after dietary supplementation with probiotics study type: a randomized clinical trial	To evaluate the capacity of a novel nutritional supplement to decrease the viral load by nasopharyngeal smear in patients admitted for COVID-19 Study type: interventional (clinical trial), randomized	Novel nutritional supplement intervention including strains *Bifidobacterium longum*, *Bifidobacterium animalis* subsp. *Lactis* and *Lactobacillus rhamnosus*, with vitamin D, zinc and selenium proves to be immune system enhancer, antioxidant and anti-inflammatory	Viral load during the period of admission to the nasopharyngeal smear	Clinical indicators like non-invasive mechanical ventilation (NIV) intervention, IL-6 initial point, hospital stay (days) and stay in Intensive Care Unit (days), Microbiome analysis in feces	ClinicalTrials.gov identifier: NCT04666116
Efficacy and safety of *Lactobacillus Plantarum* and *Pediococcus Acidilactici* as co-adjuvant therapy for reducing the risk of severe disease in adults with SARS-CoV-2 and its modulation of the fecal microbiota: a randomized clinical trial	To evaluate how combination of probiotics reduce the risk to progress to moderate or severe COVID and associated advantages such as reduce the risk of death, to explore the benefits of this combination of strains to modulate fecal microbiome and explore how this correlate with clinical improvement Study type:	Dietary supplement Probiotics: combination of *Lactobacillus plantarum* CECT7481, *Lactobacillus plantarum* CECT 7484, *Lactobacillus plantarum* CECT 7485, and *Pediococcus acidilactici* CECT 7483	Severity progression of COVID-19, Stay at ICU and Mortality ratio	Lung abnormalities, viral load, levels of immunoglobulins, gastrointestinal manifestations, fecal microbiome, adverse events, change on serum biomarkers	ClinicalTrials.gov identifier: NCT04517422

(continued)

Table 2.1 (continued)

Study title	Aim and study type	Intervention and probiotics	Primary outcome	Secondary outcome	References
	interventional (clinical trial), randomized				
Multicentric study to assess the effect of consumption of *Lactobacillus Coryniformis* K8 on healthcare personnel exposed to COVID-19	To evaluate the effects of *Lactobacillus coryniformis* K8 consumption on the incidence and severity of Covid-19 in health workers exposed to the virus Study type: interventional (clinical trial), randomized	Probiotic: *Lactobacillus coryniformis* K8	The incidence of SARS CoV-2 infection will be confirmed by PCR or antigen test	Incidence of hospital admissions, ICU admissions, pneumonia, oxygen support requirement and gastrointestinal symptoms caused by SARS-CoV-2 infection	ClinicalTrials.gov identifier: NCT04366180
Evaluation of the impact of bacteriotherapy in the treatment of COVID-19	To evaluate the effectiveness of bacteriotherapy in reducing the clinical impact of acute diarrhoea, containing the progression of COVID-19 and preventing the need for hospitalization in intensive care units Study type: observational, case-control, retrospective	Probiotic: Composition of SivoMixx: *Streptococcus thermophilus* DSM322245, *Bifidobacterium lactis* DSM 32246, *Bifidobacterium lactis* DSM 32247, *Lactobacillus acidophilus* DSM 32241, *Lactobacillus helveticus* DSM 32242, *Lactobacillus paracasei* DSM 32243,	Delta of time of disappearance of acute diarrhea	Delta[b] in the number of patients requiring orotracheal intubation despite treatment, Delta of crude mortality, Delta of length of stay for patients in hospital	ClinicalTrials.gov identifier: NCT04368351

		Lactobacillus plantarum DSM 32244, *Lactobacillus brevis* DSM[a] 27961			
Oxygen-ozone as adjuvant treatment in early control of disease progression in patients with COVID-19 associated with modulation of the gut microbial flora	To evaluate the effectiveness of an ozone therapy-based intervention (accompanied by supplementation with probiotics) in containing the progression of COVID-19 and in preventing the need for hospitalization in intensive care units Study type: Interventional (clinical trial), randomized	Probiotic: Used as dietary supplement of SivoMixx: *Streptococcus thermophilus* DSM322245, *Bifidobacterium lactis* DSM 32246, *Bifidobacterium lactis* DSM 32247, *Lactobacillus acidophilus* DSM 32241, *Lactobacillus helveticus* DSM 32242, *Lactobacillus paracasei* DSM 32243, *Lactobacillus plantarum* DSM 32244, *Lactobacillus brevis* DSM 27961	Delta in the number of patients requiring orotracheal intubation despite treatment	Delta of crude mortality, Delta of length of stay for patients in hospital, Delta in the value of interleukin IL-1, IL-6, IL-10, tumor Necrosis Factor (TNF)-alpha, cluster of differentiation for CD^{4+} and CD38	ClinicalTrials.gov identifier: NCT04366089

[a] DSM n° bacterial strain identification code
[b] DELTA (difference ELicitation in TriAls)

3. Probiotics improve mucosal immunity
4. Probiotic therapy reduces pro-inflammatory cytokines during respiratory infections.
5. Intestinal probiotics evade an overreaction of the immune system, thus maintaining immune homeostasis.
6. Probiotics such as *Lactobacillus* and *Bifidobacterium* exhibit a reduction in the gut of few COVID-19 patients (Bottari et al. 2020).

Still, more robust and significant research are required to comprehend the role of the microbiome and probiotics in COVID-19-infected patients, but the possibility persists that such research investigations can identify novel approaches to attenuate and prevent this ailment.

References

Bo L, Li J, Tao T, Bai Y, Ye X, Hotchkiss RS, Kollef MH, Crooks NH, Deng X (2014) Probiotics for preventing ventilator-associated pneumonia. Cochrane Database Syst Rev 10:CD009066

Bottari B, Castellone V, Neviani E (2020) Probiotics and Covid-19. Int J Food Sci Nutr 2020:1–7

Cani PD, de Vos WM (2017) Next-generation beneficial microbes: the case of Akkermansia muciniphila. Front Microbiol 8:1765

Cheung CY, Poon LL, Ng IH, Luk W, Sia SF, Wu MH, Chan KH, Yuen KY, Gordon S, Guan Y, Peiris JS (2005) Cytokine responses in severe acute respiratory syndrome coronavirus-infected macrophages in vitro: possible relevance to pathogenesis. J Virol 79(12):7819–7826

Coleman CM, Frieman MB (2014) Coronaviruses: important emerging human pathogens. J Virol 88(10):5209–5212

Cook MT, Tzortzis G, Charalampopoulos D, Khutoryanskiy VV (2012) Microencapsulation of probiotics for gastrointestinal delivery. J Control Release 162(1):56–67

Couzin-Frankel J (2020) The mystery of the pandemic's 'happy hypoxia'. Science 368(6490): 455–456

de Vrese M, Winkler P, Rautenberg P, Harder T, Noah C, Laue C, Ott S, Hampe J, Schreiber S, Heller K, Schrezenmeir J (2005) Effect of Lactobacillus gasseri PA 16/8, Bifidobacterium longum SP 07/3, B. bifidum MF 20/5 on common cold episodes: a double blind, randomized, controlled trial. Clin Nutr 24(4):481–491

Dermyshi E, Wang Y, Yan C, Hong W, Qiu G, Gong X, Zhang T (2017) The "golden age" of probiotics: a systematic review and meta-analysis of randomized and observational studies in preterm infants. Neonatology 112(1):9–23

Ettinger G, MacDonald K, Reid G, Burton JP (2014) The influence of the human microbiome and probiotics on cardiovascular health. Gut Microbes 5(6):719–728

Fuglsang A, Rattray FP, Nilsson D, Nyborg NC (2003) Lactic acid bacteria: inhibition of angiotensin converting enzyme in vitro and in vivo. Antonie Van Leeuwenhoek 83(1):27–34

Gibson PG, Qin L, Puah SH (2020) COVID-19 acute respiratory distress syndrome (ARDS): clinical features and differences from typical pre-COVID-19 ARDS. Med J Aust 213(2):54–56

Glowacka I, Bertram S, Müller MA, Allen P, Soilleux E, Pfefferle S, Steffen I, Tsegaye TS, He Y, Gnirss K, Niemeyer D (2011) Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. J Virol 85(9):4122–4134

Gonzalez-Gonzalez C, Gibson T, Jauregi P (2013) Novel probiotic-fermented milk with angiotensin I-converting enzyme inhibitory peptides produced by Bifidobacterium bifidum MF 20/5. Int J Food Microbiol 167(2):131–137

Gorbalenya A, Baker S, Baric R, de Groot R, Drosten C, Gulyaeva A, Haagmans B, Lauber C, Leontovich A, Neuman B, Penzar D (2020) The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol 2020: 3–4

Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DS, Du B (2020) Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 382(18):1708–1720

Hill C et al (2014) The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 11:506–514

Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA (2020) SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 181(2):271–280

Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z (2020) Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395(10223):497–506

Jia HP, Look DC, Shi L, Hickey M, Pewe L, Netland J, Farzan M, Wohlford-Lenane C, Perlman S, McCray PB (2005) ACE2 receptor expression and severe acute respiratory syndrome coronavirus infection depend on differentiation of human airway epithelia. J Virol 79(23):14614–14621

Kai H, Kai M (2020) Interactions of coronaviruses with ACE2, angiotensin II, and RAS inhibitors—lessons from available evidence and insights into COVID-19. Hypertens Res 2020:1–7

Kumar M, Al Khodor S (2020) Pathophysiology and treatment strategies for COVID-19. J Transl Med 18(1):1–9

Laursen RP, Hojsak I (2018) Probiotics for respiratory tract infections in children attending day care centers—a systematic review. Eur J Pediatr 177(7):979–994

Li H, Liu SM, Yu XH, Tang SL, Tang CK (2020a) Coronavirus disease 2019 (COVID-19): current status and future perspective. Int J Antimicrob Agents 2020:105951

Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, Ren R, Leung KS, Lau EH, Wong JY, Xing X (2020b) Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia. N Engl J Med 382(13):1199–1207

Lopez-Siles M, Duncan SH, Garcia-Gil LJ, Martinez-Medina M (2017) Faecalibacterium prausnitzii: from microbiology to diagnostics and prognostics. ISME J 11(4):841–852

Luoto R, Ruuskanen O, Waris M, Kalliomäki M, Salminen S, Isolauri E (2014) Prebiotic and probiotic supplementation prevents rhinovirus infections in preterm infants: a randomized, placebo-controlled trial. J Allergy Clin Immunol 133(2):405–413

Mason RJ (2020) Pathogenesis of COVID-19 from a cell biology perspective. Eur Respir J 55(4): 2000607

Miremadi F, Ayyash M, Sherkat F, Stojanovska L (2014) Cholesterol reduction mechanisms and fatty acid composition of cellular membranes of probiotic Lactobacilli and Bifidobacteria. J Funct Foods 9:295–305

Muthukumarasamy P, Allan-Wojtas P, Holley RA (2006) Stability of Lactobacillus reuteri in different types of microcapsules. J Food Sci 71(1):20–24

Namba K, Hatano M, Yaeshima T, Takase M, Suzuki K (2010) Effects of Bifidobacterium longum BB536 administration on influenza infection, influenza vaccine antibody titer, and cell-mediated immunity in the elderly. Biosci Biotechnol Biochem 74(5):939–945

Olaimat AN, Aolymat I, Al-Holy M, Ayyash M, Ghoush MA, Al-Nabulsi AA, Osaili T, Apostolopoulos V, Liu SQ, Shah NP (2020) The potential application of probiotics and prebiotics for the prevention and treatment of COVID-19. NPJ Sci Food 4(1):1–7

Panigrahi P, Parida S, Nanda NC, Satpathy R, Pradhan L, Chandel DS, Baccaglini L, Mohapatra A, Mohapatra SS, Misra PR, Chaudhry R (2017) A randomized synbiotic trial to prevent sepsis among infants in rural India. Nature 548(7668):407–412

Radenkovic D, Chawla S, Pirro M, Sahebkar A, Banach M (2020) Cholesterol in relation to COVID-19: should we care about it? J Clin Med 9(6):1909

Rautava S, Salminen S, Isolauri E (2008) Specific probiotics in reducing the risk of acute infections in infancy–a randomised, double-blind, placebo-controlled study. Br J Nutr 101(11):1722–1726
Rico-Mesa JS, White A, Anderson AS (2020) Outcomes in patients with COVID-19 infection taking ACEI/ARB. Curr Cardiol Rep 22:1–4
Rockx B, Baas T, Zornetzer GA, Haagmans B, Sheahan T, Frieman M, Dyer MD, Teal TH, Proll S, Van Den Brand J, Baric R (2009) Early upregulation of acute respiratory distress syndrome-associated cytokines promotes lethal disease in an aged-mouse model of severe acute respiratory syndrome coronavirus infection. J Virol 83(14):7062–7074
Shori AB (2017) Microencapsulation improved probiotics survival during gastric transit. HAYATI J Biosci 24(1):1–5
Spiegel M, Pichlmair A, Martínez-Sobrido L, Cros J, García-Sastre A, Haller O, Weber F (2005) Inhibition of beta interferon induction by severe acute respiratory syndrome coronavirus suggests a two-step model for activation of interferon regulatory factor 3. J Virol 79(4): 2079–2086
Tang NLS, Chan PKS, Wong CK, To KF, Wu AKL, Sung YM, Hui DSC, Sung JJY, Lam CWK (2005) Early enhanced expression of interferon-inducible protein-10 (CXCL-10) and other chemokines predicts adverse outcome in severe acute respiratory syndrome. Clin Chem 51(12):2333–2340
Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LF (2020) The trinity of COVID-19: immunity, inflammation and intervention. Nat Rev Immunol 2020:1–12
Tobin MJ, Laghi F, Jubran A (2020) Why COVID-19 silent hypoxemia is baffling to physicians. Am J Respir Crit Care Med 202(3):356–360
Tochio T, Kadota Y, Tanaka T, Koga Y (2018) 1-Kestose, the smallest fructooligosaccharide component, which efficiently stimulates faecalibacterium prausnitzii as well as bifidobacteria in humans. Foods 7(9):140
Waki N, Matsumoto M, Fukui Y, Suganuma H (2014) Effects of probiotic Lactobacillus brevis KB 290 on incidence of influenza infection among schoolchildren: an open-label pilot study. Lett Appl Microbiol 59(6):565–571
Wan Y, Shang J, Graham R, Baric RS, Li F (2020) Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. J Virol 94(7):e00127
Weiss SR (2020) Forty years with coronaviruses. J Exp Med 217:20200537
Wong CK, Lam CWK, Wu AKL, Ip WK, Lee NLS, Chan IHS, Lit LCW, Hui DSC, Chan MHM, Chung SSC, Sung JJY (2004) Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. Clin Exp Immunol 136(1):95–103
World Health Organization (2020) 'Solidarity' clinical trial for COVID-19 treatment. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/global-research-on-novel-coronavirus-2019-ncov/solidarity-clinical-trial-for-covid-19-treatments
Wu Z, McGoogan JM (2020) Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA 323(13):1239–1242
Xu K, Cai H, Shen Y, Ni Q, Chen Y, Hu S, Li J, Wang H, Yu L, Huang H, Qiu Y (2020) Management of corona virus disease-19 (COVID-19): the Zhejiang experience. Zhejiang Da Xue Xue Bao Yi Xue Ban 49(1):147–157
Yang D, Leibowitz JL (2015) The structure and functions of coronavirus genomic 3′ and 5′ ends. Virus Res 206:120–133
Yang L, Liu S, Liu J, Zhang Z, Wan X, Huang B, Chen Y, Zhang Y (2020) COVID-19: immunopathogenesis and immunotherapeutics. Signal Transduct Target Ther 5(1):1–8

Yao M, Xie J, Du H, McClements DJ, Xiao H, Li L (2020) Progress in microencapsulation of probiotics: a review. Compr Rev Food Sci Food Saf 19(2):857–874

Yasui H, Kiyoshima J, Hori T, Shida K (1999) Protection against influenza virus infection of mice fed Bifidobacterium breve YIT4064. Clin Diagn Lab Immunol 6(2):186–192

Youn HN, Lee DH, Lee YN, Park JK, Yuk SS, Yang SY, Lee HJ, Woo SH, Kim HM, Lee JB, Park SY (2012) Intranasal administration of live Lactobacillus species facilitates protection against influenza virus infection in mice. Antivir Res 93(1):138–143

Zhu Z, Lian X, Su X, Wu W, Marraro GA, Zeng Y (2020) From SARS and MERS to COVID-19: a brief summary and comparison of severe acute respiratory infections caused by three highly pathogenic human coronaviruses. Respir Res 21(1):1–14

3 Plant-Based Bioactive Compounds in Cancer Therapeutics

Neha Jain, Pallavi Kaushik, Aparna Pareek, Hemant Pareek, and Mukesh Kumar Sharma

Abstract

Cancer is a dreadful disease, and it is considered as one of the biggest health problems for the human populations and demands an intense strategy to cure. Cancer causes around 70% death in lower- and middle-income countries. Recent studies have reported that natural bioactive compounds (obtained from medicinal plants) in combination with anticancer drugs have great potential to destroy cancerous cells while not affecting normal cells. Natural bioactive compounds are considered suitable candidates for anticancer drug development with the great success opportunity due to their excellent properties for modulating multiple cellular and molecular targets of oncogenesis directly or indirectly and have shown great progress past several decades. The chief anticancerous properties include the inhibition of tumor cell growth, the induction of apoptosis, DNA damage, the inhibition of topoisomerases I and II, and others. Nowadays, many researchers are on the way to develop potential drugs that can inhibit or delay the growth of tumor cells without any type of side effects from natural bioactive compounds. The chief plant-based bioactive compounds with potential cancer therapeutic potential include curcumin, camptothecin, diosgenin, epigallocatechin-3-gallate, homoharringtonine, piperine, podophyllotoxin, resveratrol, vinca alkaloids, etc.

N. Jain · P. Kaushik
Centre for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur, India

A. Pareek
Department of Botany, University of Rajasthan, Jaipur, India

H. Pareek
Department of College Education, Government of Rajasthan, Jaipur, India

M. K. Sharma (✉)
Department of Zoology, SPC Government College, Ajmer, India

K. K. Behera et al. (eds.), *Prebiotics, Probiotics and Nutraceuticals*,
https://doi.org/10.1007/978-981-16-8990-1_3

This chapter provides a comprehensive report on the recent advancements and breakthrough accomplished in cancer treatments using natural bioactive compounds alone or in combination with other known therapies along with their possible mechanism of action on nuclear and cellular factors.

Keywords

Bioactive compounds · Anticancer therapeutics · Apoptosis · Topoisomerase inhibition

3.1 Introduction

One of the leading causes of death worldwide is the debilitating disease cancer in which certain cell loses the control on division leading to the unwanted overgrowth or tumors. As per the IARC (International Agency for Research on Cancer), the worldwide mortality due to cancer in 2018 was 9,555,027 and about 18,078,957 new cancer cases. Maximum mortality was witnessed in Asia followed by Europe, North America, Africa, Latin America and the Caribbean, and Oceania. The WHO has also launched Global Action Plan for the prevention and control of noncommunicable diseases 2013–2020 which aims at reducing the premature mortality against noncommunicable diseases including cancer.

It not only disrupts the host organ but can also disturb or destruct the nearby tissues and organs by invasion or metastasis. Some tumors become confined to their original location and are composed of cells with normal physicalities; these are called benign tumors which are comparatively easy to manage by its removal (Sinha 2018). These are generally noncancerous but can be harmful if they press upon nearby nerves, blood vessels, or vital organs. Some of these are also considered precancerous; thus, immediate removal is generally recommended. The other category includes the spread of abnormal tumor cells from the original site not only to the nearby site but also to distant locations throughout the body by the migration of tumor cells through the general body circulation. This condition is referred to as tumor malignancy which is much complex with doubtful therapeutic success. Although a clear demarcation between benign as well as malignant tumor is seldom observed (Thielker et al. 2018). There are many instances when the most complex cancers are managed successfully using combinational therapies including the traditional radiotherapy and chemotherapy along with the use of certain compounds with therapeutic potential.

The twenty-first-century therapeutics includes surgery, radiotherapy, chemotherapy, immunotherapy, and even hormonal therapy. Surgery is the basic method which involves the excision of tumor along with nearby tissue which can cause local pathological formations along with the risk of metastasis. About 50% of cancer patients receive radiotherapy involving ionizing radiations. They are administered either externally or internally at low, high, or fractional doses with or without adjuvants which are aimed at killing the cancer cells by damaging its genetic material. Radiotherapy is often injurious to the normal cells or nontarget cells (Baskar et al. 2012). Chemotherapy causes cytotoxicity to the specific tumor cells as these are generally chemical agents with antimitotic capacity. This therapy provides comparatively successful treatment (Theilen et al. 1987) along with combination therapies to provide better life quality and increased life span in patients.

The abovementioned therapies are either invasive or toxic to the cancer cells as well as the healthy nontarget cells. The injury caused to nontarget cells takes a long time to heal and renders the patient in extreme morbidity in post-therapy period. The newer approach is biocontrol or biotherapies, which involves biological substances which can destroy the target cancer cells. This therapy can consist of cellular activity or molecular activity. Immunotherapy against cancer is one of the strategies in which customized cells (T Cells) or target-specific molecules (monoclonal antibodies) are used against tumor antigens resulting in cytotoxic response. But in many cases, tumor tolerance and eventual tumor escape are also observed. Currently the immunotherapy also involves the use of cytokines, interferons, and even vaccines to prevent cancer development (Jiang and Zhou 2015).

The plant-based natural bioactive compounds have been extensively studied as anticancer agent which can act at various levels including prevention of invasion and metastasis (Cicerale et al. 2010; Wang et al. 2012; Luo et al. 2014). There is great body of investigations on such bioactive compounds which can provide protection against various cancers types.

The chapter shall encompass an array of plant-based bioactive compounds like curcumin, camptothecin, diosgenin, epigallocatechin-3-gallate, homoharringtonine, piperine, podophyllotoxin, resveratrol, vinca alkaloids, lycopenes, anthocyanins, apigenins, quercetin, isothiocyanates, ursolic acid, withaferin A, etc., which have been studied for their anticancer properties and even used in standard therapies.

3.2 Drawbacks of Standard Therapies

The standard therapies currently used for treatment of cancer give rise to lifelong morbidities and lot of complications like imposing bystander effects, early relapses, multidrug resistance, high cost, low efficacy, and several other lethal side effects (Demain and Vaishnav 2011; Prakash et al. 2013; Block et al. 2015; Samadi et al. 2015; Feitelson et al. 2016; Badawy et al. 2018). So, there is an imperative need for devising health promising strategies with naturally occurring bioactive compounds (often used as functional dietary food) as alternative novel therapeutic agents.

3.3 Potential of Natural Bioactive Compounds in Cancer Therapeutics

Natural bioactive compounds have the potential for being anticancer agents with high-priority objective and low toxicity and can even target multiple drug resistance and heterogeneity of the tumor cells showing high efficacy with low cost and easy availability giving safe and high-quality life with less or no side effects (Demain and Vaishnav 2011; Prakash et al. 2013; Block et al. 2015; Samadi et al. 2015; Feitelson et al. 2016; Harsha Raj et al. 2017; Badawy et al. 2018; De Silva and Alcorn 2019; Oyeyinka and Afolayan 2019). Natural products and their derivatives act on the life-threatening disease by interfering with the mechanism of the carcinogenesis and its multistep progression, preventing proliferation by one or more pathways, and also by arresting cell cycle, modulating and even inhibiting metastasis (Harsha Raj et al. 2017; Farooq et al. 2019), inducing apoptosis, cleaving DNA, permeabilizing the mitochondrial membrane (Demain and Vaishnav 2011), and inhibiting angiogenesis (Harsha Raj et al. 2017).

3.4 Bioactive Compounds

Natural bioactive compounds from plants are attracting broad recognition for their anticancer activities. Numerous findings have shown that natural plant-derived compounds can enhance the effectiveness of various cancer therapies and in some cases alleviate many of the side effects of chemotherapeutic agents. Some of the important bioactive compounds with the potential use in cancer therapeutics are described in the following text:

1. Curcumin: Curcumin (diferuloylmethane) is a polyphenolic compound extracted from the rhizome of *Curcuma longa* (Zingiberaceae) which is a tropical Southeast Asian plant. It is attributed to broad spectrum of pharmacological activities for decades like antioxidant, anti-inflammatory, chemotherapeutic, chemopreventive, and chemo-sensitizing activity (Madka and Rao 2013; Sun et al. 2015; Seca and Pinto 2018; Khan et al. 2019; Ashraf 2020; Choudhari et al. 2020). Researchers reported anticancer properties of curcumin on in vivo and in vitro models against breast, prostate, ovarian, pancreatic, colorectal, skin, and blood cancer and hematological malignancies (Madka and Rao 2013; Choudhari et al. 2020). It gives anti-inflammatory effects and acts as anticancer chemical by inhibiting enzymes such as cyclooxygenase (COX)-2, 5-LOX, xanthine oxidase, and iNOS. Other antitumor mechanisms include cytochrome P450 enzyme modulation; caspase activation; cell cycle arrest at G1, S, and G2/M phase checkpoints; upregulation of CDKIs; inhibition of NF-κB transcription factor; and downregulation of vascular endothelial growth factor, platelet-derived growth factor, and fibroblast growth factor expression (Sun et al. 2015; Seca and Pinto 2018). Although it works as chemosensitizer with some anticancer drugs like gemcitabine, paclitaxel, 5-fluorouracil, and doxorubicin and works synergistically with natural drugs like resveratrol, honokiol, epigallocatechin-3-gallate, licochalcone, and omega-3, the compound has very low absorptivity, is improperly metabolized, and shows poor systemic bioavailability (Seca and Pinto 2018).
2. Camptothecin: Camptothecin are quinolone alkaloids isolated from *Camptotheca acuminata* which is a Chinese ornamental plant. The compound

and its derivatives pertain to antineoplastic properties (Choudhari et al. 2020; Lichota and Gwozdzinski 2018; Prakash et al. 2013) such as complexing with DNA topoisomerase 1 and inhibiting DNA's religation and cleavage, thereby causing cytotoxicity and DNA break (Choudhari et al. 2020; Lichota and Gwozdzinski 2018; Prakash et al. 2013). Currently, topotecan and irinotecan that are semisynthetic derivatives are clinically approved for the treatment of small cell lung cancer, recurring ovarian cancer, cervical cancer, and rectum and large intestine cancers, respectively, with less toxicity than the original compound (Prakash et al. 2013; Lichota and Gwozdzinski 2018; Choudhari et al. 2020).

3. Diosgenin: Diosgenin is the bioactive compound present in *Trigonella foenum-graecum* that is responsible for curing hypercholesterolemia, diabetes, gastrointestinal ailments, and cancers. Basically, it has the capability to induce apoptosis and attenuate proliferation and inflammation. It has been investigated in treating several cancer cells including Ehrlich ascites carcinoma, hepatocellular carcinoma cells, colon cancer cell, human osteosarcoma 1547 cell line, and colorectal cancer HT-29 cell lines. Basically, it affects the expression of p21 and p53 (proapoptotic proteins), suppresses various pathways such as STAT3 and MAPK, and lowers the index of proliferating cell nuclear antigen (PCNA) in some cancers. The function of chronic inflammation in oncogenesis is crucial; hence, the research of Yamada et al. in 1997 demonstrated the diosgenin's potential to heal inflammation adequately may be extended to its prospective chemopreventive action (Yamada et al. 1997; Madka and Rao 2013).
4. Epigallocatechin-3-gallate: EGCG is a chief polyphenolic component of *Camellia sinensis* having great antioxidant potential (Ahmad et al. 2000; Prakash et al. 2013; Sun et al. 2015). As per anticancerous activity, the catechin has been recorded to effectively defend against human epidermoid carcinoma (A431) cells (Ahmad et al. 2000), oral cancer cells, ovarian carcinoma (OVCA) and HEY cell lines, and colon and rectal cancer (HCA-7 and HT-29) cell lines (Prakash et al. 2013) via cell cycle arrest, apoptosis, alteration in CKI and CDK functions (Ahmad et al. 2000), and suppression of invasion and metastasis of cells (Prakash et al. 2013). Mechanistically, EGCG significantly upregulates p18, p16, KIP1/p27, and WAF1/p21 expressions and downregulates Upa, MMP-9, MMP-2, cdk4, cdk6 and cyclin D1, NF-κB, HIF-1α, and VEGF expressions, making it as a persuasive antitumor agent (Ahmad et al. 2000; Prakash et al. 2013; Sun et al. 2015).
5. Homoharringtonine: Homoharringtonine is an ester of cephalotaxine obtained from *Cephalotaxus harringtonia* and is known to treat chronic myeloid leukemia (Prakash et al. 2013; Lichota and Gwozdzinski 2018; Choudhari et al. 2020) and breast cancer (Lichota and Gwozdzinski 2018). Moreover, the compound has been verified to deliver absolute hematologic diminution in late chronic stage, chronic myelogenous leukemia patients (Prakash et al. 2013). Mechanistically, it trusses to A-site cleft of the large ribosomal subunit, thereby blocking the access of charged tRNA and ultimately inhibiting translation process leading to apoptosis (Prakash et al. 2013; Lichota and Gwozdzinski 2018; Choudhari

et al. 2020). In patients with aversion and resistance to hypomethylating agents, a homoharringtonine semisynthetic derivative omacetaxine mepesuccinate has proven to be effective for chronic myelomonocytic leukemia and myelodysplastic syndromes (Choudhari et al. 2020) making the compound a prominent therapeutic agent.

6. Piperine: Piperine is an alkaloid obtained from *Piper nigrum* that has been recognized for numerous advantageous properties, namely, improving digestive capability and dropping the passage period of gastrointestinal food. In addition, by suppressing or tempering relative oxygen species and reducing lipid peroxidation, piperine has shown to protect against oxidative damage, thereby serving as an anti-inflammatory and antitumorigenic bioactive source. Its anticancerous potential has been evaluated in melanoma, lung cancer, sarcoma, and colon cancer through cytokine reduction, decreased metastasis, polyamine synthesis, lipid peroxidation, and alterations of many pathways. Furthermore, when used in combination with other substances, piperine not only enhances their bioactivity but also increases their effectiveness. For instance, a combination of piperine and curcumin in a study dramatically reduced the amount of DNA break and 8-oxo-dG concentration in BP-persuaded DNA damage in the livers, lungs, and colon of mice, suggesting an improved geno-protective capacity compared to curcumin solely (Madka and Rao 2013).
7. Podophyllotoxin: A bioactive compound known for anticancer properties against skin cancers is extracted from *Podophyllum emodi* and *Podophyllum peltatum* (Prakash et al. 2013; Lichota and Gwozdzinski 2018; Choudhari et al. 2020). The compound directly disrupts the karyokinetic spindle organization by binding to tubulin and suppressing its activity in reversible manner (Prakash et al. 2013; Lichota and Gwozdzinski 2018; Choudhari et al. 2020). In addition, through their encounters with DNA topoisomerase II, the podophyllotoxins induce double- and single-stranded breaks in DNA causing G2 phase cell cycle arrest. Some of the semisynthetic derivatives, namely, teniposide, etoposide, etopophos, azatoxin, tafluposide, NK61, GL33, and TOP-53, also possess anti-neoplasticity by affecting the metaphase of the cell cycle ultimately leading to apoptosis (Lichota and Gwozdzinski 2018). Also, these compounds display anti-multidrug resistance ability against multiple tumor cells. For example, by controlling the activity of topoisomerase-IIa, a derivative known as CIP-36 has been reported to resolve the multidrug resistance of cell line K562/ADR of adriamycin-resistant human leukemia (Choudhari et al. 2020). Podophyllotoxin and its derivatives have been efficiently explored for its anticancer potential against neuroblastomas, carcinomas, sarcomas, lymphomas, gliomas, and testicular, ovarian, cervical, colon, lung, breast, gastrointestinal, prostate, and brain cancers (Lichota and Gwozdzinski 2018).
8. Resveratrol: Resveratrol (trans-3,5,4′-trihydroxystilbene) is a natural polyphenol that belongs to astilbenoid compounds and primarily extracted from red grapes, cranberries, peanuts, blueberry, mulberry, etc. (Madka and Rao 2013; Sun et al. 2015; Choudhari et al. 2020). It includes several beneficial aspects comprising antiestrogenic and antioxidative activities and capacity to decrease

eicosanoid and hepatic lipid synthesis. It also prevents accumulation of platelets and defends vessels from arteriosclerosis (Sun et al. 2015). The impending ability of antitumorigenesis has been studied in esophageal, pancreatic, melanoma, thyroid, ovarian, prostate, lung, colon, gastric, liver, endometrial, head, and neck cancers as well as colon cancer cell lines such as Caco-2, HCT 116, and HT29. Resveratrol's inhibitory properties have been shown to be essential in order to alter a diverse spectrum of cellular components and proliferation contributing pathways. Moreover, LOX, iNOS, and COX2 and other inflammatory molecules are also suppressed (Madka and Rao 2013). In addition, the phytochemical has dual role on cells, depending on the condition and cell types, i.e., it can either cause or inhibit angiogenic effects demonstrating activity of antiproliferation in separate tumor cells (Sun et al. 2015).

9. Taxanes: This is a class of molecules that are derived from plants known for inhibiting mitosis. Paclitaxel also known as Taxol is a bioactive compound extracted from *Taxus brevifolia*. Its semisynthetic derivatives cabazitaxel (CTX) and docetaxel (DTX) have been successfully explored in the breast, pancreas, ovarian, lung, and Kaposi sarcoma, head and neck carcinoma, and prostate cancer monotherapies as well as in amalgamation with other anticancer agents (Prakash et al. 2013; Lichota and Gwozdzinski 2018). With improved cytotoxicity in resistant tumors, reduced toxicity, and enhanced solubility, a multitude of semisynthetic derivatives have been generated. For instance, cabazitaxel is a second-generation docetaxel analogue which displays cytotoxic activity with less cumulative exposure against multiple docetaxel-resistant cancer cells. Moreover, cabazitaxel has the propensity to cross the blood-brain barrier that cannot be accomplished with other taxanes. Additionally, some of the paclitaxel analogues are currently under clinical review comprising tesetaxel, ortataxel, milataxel, and larotaxel (Prakash et al. 2013; Lichota and Gwozdzinski 2018). Anticancer mechanism involves direct association with microtubules and their stabilizing mediators leading to their polymerization (Lichota and Gwozdzinski 2018). The significance of taxanes can be assessed by the conclusion that preclinical or clinical expansion involves more than a dozen analogues. Furthermore, the National Cancer Institute have documented 2069 cancer clinical trials, out of which 248 are mentioned as implicating taxane derivatives including 134 with paclitaxel, 105 with docetaxel, and 10 with taxane-derived assorted drugs in clinical research (Prakash et al. 2013).
10. Vinca Alkaloids: Vinca alkaloids also called as *Catharanthus* alkaloids extracted from the *Catharanthus roseus* were the first agents that were introduced in health sector. It includes 130 terpenoid indole alkaloids, among which vinblastine was the first to be isolated (Lichota and Gwozdzinski 2018). The major alkaloids comprise of vinblastine, vincristine, anhydrovinblastine, and some semisynthetic derivatives such as vinflunine, vindesine, and vinorelbine (Prakash et al. 2013; Lichota and Gwozdzinski 2018; Choudhari et al. 2020). *Catharanthus* alkaloids exhibit cytotoxic effects on cancerous cells by targeting microtubules and binding to its tubulin subunits. Apparently, based on their concentration, the vinca alkaloids show distinct action mechanisms as at

low concentration (<1 μmol), these suppress and maintain the dynamics of the microtubules, whereas at high concentrations (>1–2 μmol), these directly collapse the microtubules and disrupt the mitotic spindle, contributing to mitosis inhibition and apoptosis (Lichota and Gwozdzinski 2018; Choudhari et al. 2020). For the prevention of non-small cell lung, bladder, renal, ovarian, and breast cancers, Kaposi sarcoma, and soft tissue sarcoma, alkaloids are employed in chemotherapy. They are also known to treat malignant lymphomas, acute lymphocytic leukemias, idiopathic thrombocytopenic purpura, and various solid tumors, namely, retinoblastoma, Wilms tumor, Ewing's sarcoma, and neuroectodermal tumors (Prakash et al. 2013; Lichota and Gwozdzinski 2018; Choudhari et al. 2020).

More than 160 of over 2069 therapeutic investigations identified by the National Cancer Institute for drug formulations use these mediators against a wide range of cancers (Prakash et al. 2013) (Figs. 3.1, 3.2, and 3.3).

Other major bioactive compounds that possess inherent property to act against various cancers are lycopenes, anthocyanins, apigenins, quercetin, isothyocyanates, ursolic acid, withaferin A, etc. (Table 3.1) from the specific medicinal plants.

3.5 Bioactive Compounds and Their Derivatives as Approved Drug Against Cancer

Nowadays, many researchers are on the way to develop potential drugs that can inhibit or delay the growth of tumor cells without any type of side effects from natural bioactive compounds. Velban® and Oncovin® are the trade names under which vinblastine and vincristine, respectively, have been approved as therapeutic agents against lymphomas and solid tumors by the FDA in 1960. Similarly, Camptosar®, having irinotecan, was permitted for colorectal cancer in 1996 where as Taxol® is approved for paclitaxel in opposition to ovarian, prostate, breast, and solid tumors in 1992 and its liposomal formulation under the trade name of Lipusu® in the year 2006. Other taxane drugs include CT-2103, Xyotax™, Taxoprexin in phases II and III, XPR9881 in phases I–III, BIND-014 in phases I and II, and CriPec® in phase I with Abraxane®. Hycamtin® has also been supported by the FDA in 2007 for small cell lung cancer with Topotecan as the content, but in 2011, it was also approved for cervical cancer with cisplatin in amalgamation. Additionally, in 2009, the FDA approved Javlor® with vinflunine for the treatment of urothelial carcinoma. In the year 2012, Picato® with ingenol mebutate formulated in gel was approved by the FDA as well as the EMA. Moreover, omacetaxine mepesuccinate under the name of Synribo® against myeloid leukemia (Seca and Pinto 2018; Sharifi-rad et al. 2019) and (SM/Chol) liposomal vincristine under the name of Marqibo® against acute lymphoblastic leukemia were accepted in 2012 and Kadcyla® in 2013 for the treatment of HER2-positive breast cancer having ado-trastuzumab emtansine as the major component (Seca and Pinto 2018). Other drugs which are clinically approved lately in the cancer therapeutics comprise of Meriva®, Teavigo™

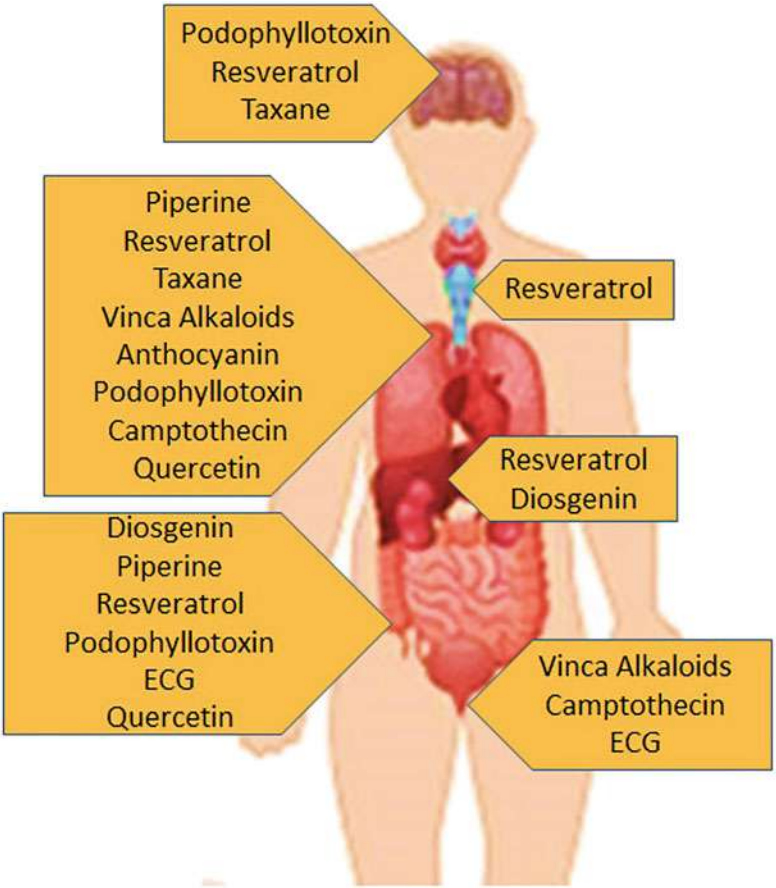


Fig. 3.1 This is an indicative description of the potential therapeutic use of the various bioactive compounds on the cancer of target organ, viz., the brain, esophagus, liver, intestine, and rectal and renal tissues

(Choudhari et al. 2020), cabazitaxel (Jevtana), eribulin (Halaven), rucaparib (Rubraca), niraparib (Zejula), talazoparib (Talzenna), pomalidomide (Pomalyst), Lenalidomide (Revlimid), erdafitinib (Balversa), and lenvatinib (Lenvima) (Sharifi-Rad et al. 2019).

3.6 Discussion and Conclusion

In developed and emerging countries, cancer has now become a high-profile illness, and its management is a struggle in some promising cases. The drugs which are synthesized and used in standard treatments, conversely, have large shortcomings

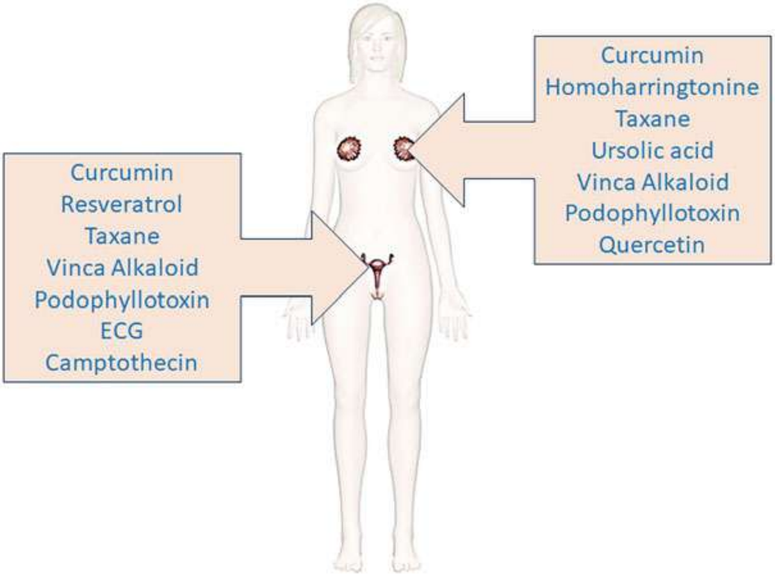


Fig. 3.2 Showing the potential therapeutic use of the various bioactive compounds on breast and cervical cancer

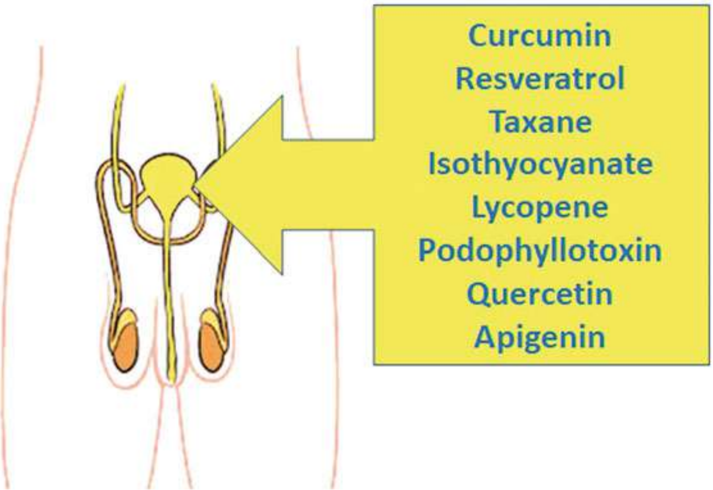


Fig. 3.3 Indicating the bioactive compounds found efficacious in prostate cancer

because of their adverse effects on nontarget cells paving way toward various health problems. Consequently, the demand for substitutes, i.e., naturally occurring agents, has been taken into consideration. In sum, we contemplated the usage of natural

Table 3.1 Bioactive compounds with their mechanisms of action against various cancers

S. no.	Bioactive compounds	Source	Type of cancer	Mechanisms of action	References
1	Curcumin (diferuloylmethane)	Golden spice *Curcuma longa*	Breast, prostate, ovarian, pancreatic, colorectal, skin, and blood cancer	Anti-inflammatory, induce apoptosis via p53, dysfunction proliferation, affect cell cycle checkpoints	(Madka and Rao 2013; Samadi et al. 2015; Sun et al. 2015; Feitelson et al. 2016; Seca and Pinto 2018; Khan et al. 2019; Ashraf 2020; Choudhari et al. 2020)
2	Resveratrol (3,5,4′-trihydroxystlbene)	Skin of red grapes, red wine, berries, peanut, etc.	Esophageal, pancreatic, melanoma, thyroid, ovarian, prostate, lung, colon, gastric, liver, endometrial, head, and neck cancers	Anti-inflammatory, prevent DNA damage, induce apoptosis via p53	(Madka and Rao 2013; Samadi et al. 2015; Sun et al. 2015; Feitelson et al. 2016; Choudhari et al. 2020)
3	Epigallocatechin gallate (EGCG)	White, green, and black tea	Epidermoid carcinoma, oral cancer, ovarian carcinoma, colon, and rectal cancer	Conceal proliferation, induce apoptosis, promote tumor suppressors, and inhibit group of cytokines	(Ahmad et al. 2000; Prakash et al. 2013; Samadi et al. 2015; Sun et al. 2015; Feitelson et al. 2016)
4	Lycopene (from the family of carotenoids)	Fruits and vegetables like tomato, apricot, guava, watermelon, pink grapefruit	Prostate and colon cancer	Antioxidant, decrease malignancy, anti-inflammatory, immune suppression	(Pouchieu et al. 2014; Samadi et al. 2015; Feitelson et al. 2016; Ashraf 2020; Choudhari et al. 2020; Omara et al. 2020)
5	Anthocyanins	Black currant skin, berries	Lung cancer	Downregulation of protein oxidation, iNOS NF-KB, COX-2,3-NT, anti-inflammatory	(Samadi et al. 2015; Feitelson et al. 2016)
6	Genistein (GEN)	An isoflavin	Bladder cancer	Induce AKT induce apoptosis, inhibit proliferation	(Samadi et al. 2015; Sun et al. 2015; Feitelson et al. 2016)
7	Apigenin	Flavone plant	Prostate cancer	Antioxidant, antimutagenic,	(Samadi et al. 2015; Feitelson et al. 2016)

(continued)

Table 3.1 (continued)

S. no.	Bioactive compounds	Source	Type of cancer	Mechanisms of action	References
				antiproliferative, and anti-inflammatory	
8	Quercetin (flavonoid)	Apples, berries, grapes, onions, brassica vegetables, seeds, bark, leaves	Prostate, cervical, lung, breast, and colon cancer	Antioxidant	(Samadi et al. 2015; Sun et al. 2015; Feitelson et al. 2016)
9	Isothyocyanates	Cruciferous vegetables	Prostate cancer	Detoxification of carcinogens	(Samadi et al. 2015; Feitelson et al. 2016)
10	Ursolic acid	Medicinal plants, apple peel	Lungs, uterus, ovary, liver, stomach, rectum, colon, and brain cancer	Antioxidant	(Rajesh et al. 2015; Ashraf 2020; Omara et al. 2020)
11	Vinca alkaloids vinblastine, vincristine, serpentines	Madagascar periwinkle plant *Catharanthus*	Non-small cell lung, bladder, renal, ovarian, breast cancer, Kaposi sarcoma, and soft tissue sarcoma	Alkaloid biosynthesis	(Demain and Vaishnav 2011; Prakash et al. 2013; Lichota and Gwozdzinski 2018; Seca and Pinto 2018; Ashraf 2020; Choudhari et al. 2020)
12	Camptothecin	Angiosperms, endophytic fungus	Small cell lung cancer, recurring ovarian cancer, cervical cancer, and rectum and large intestine cancers	Act on DNA topoisomerase 1	(Demain and Vaishnav 2011; Prakash et al. 2013; Lichota and Gwozdzinski 2018; Choudhari et al. 2020)
13	Taxanes Taxol (paclitaxel)	*Taxus brevifolia*	Breast, pancreas, ovarian, lung, Kaposi sarcoma, head and neck carcinoma, and prostate cancer	Anti-proliferative, inhibit microtubule depolymerization, enhance tubulin polymerization	(Demain and Vaishnav 2011; Prakash et al. 2013; Lichota and Gwozdzinski 2018; Seca and Pinto 2018)

14	Podophyllotoxin etoposide, teniposide	Mayapple plant's roots *Podophyllum emodi* and *Podophyllum peltatum*	Neuroblastomas, carcinomas, sarcomas, lymphomas, gliomas, testicular, ovarian, cervical, colon, lung, breast, gastrointestinal, prostate, and brain cancers	Inhibit DNA topoisomerase 2	(Demain and Vaishnav 2011; Prakash et al. 2013; Lichota and Gwozdzinski 2018; Choudhari et al. 2020)
15	Naphthoquinone (shikonin pigment)	*Lithospermum erythrorhizon*	Lung, colon, and breast cancer	–	(Demain and Vaishnav 2011)
16	Homoharringtonine	*Cephalotaxus harringtonia*	Myeloid leukemia and breast cancer	Inhibit translation process and apoptosis	(Prakash et al. 2013; Lichota and Gwozdzinski 2018; Seca and Pinto 2018; Choudhari et al. 2020)
17	Diosgenin	*Trigonella foenum-graecum*	Ehrlich ascites carcinoma, hepatocellular carcinoma cells, colon cancer cell, human osteosarcoma	Induce apoptosis and attenuating proliferation and inflammation	(Madka and Rao 2013)
18	Piperine	*Piper nigrum*	Melanoma, lung cancer, sarcoma, and colon cancer	Anti-inflammatory, against oxidative damage	(Madka and Rao 2013)
19	Oleanolic acid	Olive oil, garlic	Ovary, breast, oral, and colon cancer	DNA fragmentation, inhibition of MMPs	(Madka and Rao 2013)
20	Lupeol (Fagarsterol)	Strawberry, olive, white cabbage, mangoes, green pepper, grapes, figs	Breast, ovarian, pancreatic, colon, stomach, colon, renal, and bladder cancer	Inhibit inflammation	(Madka and Rao 2013)
21	Withaferin A	*Withania somnifera*	Cervical cancer	Reduce tumor	(Prakash et al. 2013; Ashraf 2020)

organic products in combating cancer and its consequences where we mainly focused on bioactive compounds and their derivatives ubiquitously expressed in some peculiar medicinal plants. These metabolites exhibit a range of biological properties like anticancer, anti-inflammatory, antioxidant, antiangiogenic, antimicrobial, antiviral, etc. presenting as promising agents for therapeutic intervention in tumorigenesis. The influence of particular bioactive compound, however, reflects on their association with a variety of pro-oncogenic or anti-oncogenic intermediaries and their efficacy in controlling specific targets. They exhibited antitumor activity in various animal models and cell lines with less or no side effects. Current discussion demonstrated natural products as an essential asset for better perspective toward research and development and future success of pharmaceutical industries solely or in combination with conventional therapies.

Acknowledgment The authors extend their gratitude to the CSIR, New Delhi, for providing financial assistance in the form of CSIR-JRF to Neha Jain (File no. 09/149(0753)/2019-EMR-1).

References

Ahmad N, Cheng P, Mukhtar H (2000) Cell cycle dysregulation by green tea polyphenol. Biochem Biophys Res Commun 334:328–334. https://doi.org/10.1006/bbrc.2000.3297

Ashraf MA (2020) Phytochemicals as potential anticancer drugs: time to ponder nature's bounty. Biomed Res Int 2020:8602879. https://doi.org/10.1155/2020/8602879

Badawy AA, El-Magd MA, AlSadrah SA (2018) Therapeutic effect of camel milk and its exosomes on MCF7 cells in vitro and in vivo. Integr Cancer Ther 17(4):1235–1246. https://doi.org/10.1177/1534735418786000

Baskar R et al (2012) Cancer and radiation therapy: current advances and future directions. Int J Med Sci 9(3):193–199. https://doi.org/10.7150/ijms.3635

Block K et al (2015) A broad-spectrum integrative prevention design for cancer prevention and therapy. Semin Cancer Biol 35(Suppl):S276–S304. https://doi.org/10.1016/j.semcancer.2015.09.007

Choudhari AS et al (2020) Phytochemicals in cancer treatment: from preclinical studies to clinical practice. Front Pharmacol 10:1614. https://doi.org/10.3389/fphar.2019.01614

Cicerale S, Lucas L, Keast R (2010) Biological activities of phenolic compounds present in virgin olive oil. Int J Mol Sci 11(2):458–479. https://doi.org/10.3390/ijms11020458

De Silva SF, Alcorn J (2019) Flaxseed lignans as important dietary polyphenols for cancer prevention and treatment: chemistry, pharmacokinetics, and molecular targets. Pharmaceuticals 12(2):68. https://doi.org/10.3390/ph12020068

Demain AL, Vaishnav P (2011) Natural products for cancer chemotherapy. Microb Biotechnol 4(6):687–699. https://doi.org/10.1111/j.1751-7915.2010.00221.x

Farooq A et al (2019) Natural products against cancer: review on phytochemicals from marine sources in preventing cancer. Saudi Pharm J 27(6):767–777. https://doi.org/10.1016/j.jsps.2019.04.013

Feitelson MA et al (2016) Sustained proliferation in cancer: therapeutic targets. Semin Cancer Biol 35:25–54. https://doi.org/10.1016/j.semcancer.2015.02.006.Sustained

Harsha Raj M et al (2017) Suppression of VEGF-induced angiogenesis and tumor growth by Eugenia jambolana, Musa paradisiaca, and Coccinia indica extracts. Pharm Biol 55(1): 1489–1499. https://doi.org/10.1080/13880209.2017.1307422000

Jiang T, Zhou C (2015) The past, present and future of immunotherapy against tumor. Trans Lung Cancer Res 4(3):253–264. https://doi.org/10.3978/j.issn.2218-6751.2015.01.06

Khan T et al (2019) Anticancer plants: a review of the active phytochemicals, applications in animal models, and regulatory aspects. Biomol Ther 10(1):47. https://doi.org/10.3390/biom10010047
Lichota A, Gwozdzinski K (2018) Anticancer activity of natural compounds from plant and marine environment. Int J Mol Sci 19(11):3533. https://doi.org/10.3390/ijms19113533
Luo KW et al (2014) Green tea (Camellia sinensis) extract inhibits both the metastasis and osteolytic components of mammary cancer 4T1 lesions in mice. J Nutr Biochem 25(4): 395–403. https://doi.org/10.1016/j.jnutbio.2013.11.013
Madka V, Rao CV (2013) Anti-inflammatory phytochemicals for chemoprevention of colon cancer. Curr Cancer Drug Targets 13(5):542–557. https://doi.org/10.2174/15680096113139990036
Omara T, Kiprop AK, Ramkat RC, Cherutoi J, Kagoya S, Nyangena DM, Tebo TA, Nteziyaremye P, Karanja LN, Jepchirchir A, Maiyo A, Kiptui BJ, Mbabazi I, Nakiguli CK, Nakabuye BV, Koske MC (2020) Medicinal plants used in traditional management of cancer in Uganda: a review of ethnobotanical surveys phytochemistry and anticancer studies. Evid Based Complement Alternat Med 20201-26. https://doi.org/10.1155/2020/3529081
Oyeyinka BO, Afolayan AJ (2019) Comparative evaluation of the nutritive, mineral, and antinutritive composition of Musa sinensis L. (Banana) and Musa paradisiaca L. (Plantain) fruit compartments. Plan Theory 8(12):598. https://doi.org/10.3390/plants8120598
Pouchieu C, Galan P, Ducros V, Latino-Martel P, Hercberg S, Touvier M (2014) Plasma carotenoids and retinol and overall and breast cancer risk: a nested case-control study. Nutr Cancer 66(6):980–988. https://doi.org/10.1080/01635581.2014.936952
Prakash O et al (2013) Anticancer potential of plants and natural products: a review. Am J Pharm Sci 1:104–115. https://doi.org/10.12691/ajps-1-6-1
Rajesh E, Sankari LS, Malathi L, Krupaa JR (2015) Naturally occurring products in cancer therapy. J Phar Bioallied Sci 7(5):183. https://doi.org/10.4103/0975-7406.155895
Samadi AK et al (2015) A multi-targeted approach to suppress tumor-promoting inflammation. Semin Cancer Biol 35:S151–S184. https://doi.org/10.1016/j.semcancer.2015.03.006
Seca A, Pinto D (2018) Plant secondary metabolites as anticancer agents: successes in clinical trials and therapeutic application. Int J Mol Sci 19(1):263. https://doi.org/10.3390/ijms19010263
Sharifi-Rad J et al (2019) Natural products and synthetic analogs as a source of antitumor drugs. Biomol Ther 9(11):679. https://doi.org/10.3390/biom9110679
Sinha T (2018) Tumors: benign and malignant. Cancer Ther Oncol Int J 10(3):555790. https://doi.org/10.19080/CTOIJ.2018.10.555790
Sun Q, Heilmann J, König B (2015) Natural phenolic metabolites with anti-angiogenic properties - a review from the chemical point of view. Beilstein J Org Chem 11:249–264. https://doi.org/10.3762/bjoc.11.28
Theilen GH, Madewell BR, Carter SK (1987) Chemotherapy. In: Theilen GH, Madewell BR (eds) Veterinary cancer medicine. Lea & Febiger, Philadelphia, pp 157–166
Thielker J et al (2018) Contemporary management of benign and malignant parotid tumors. Front Surg 5:39. https://doi.org/10.3389/fsurg.2018.00039
Wang N et al (2012) Ellagic acid, a phenolic compound, exerts anti-angiogenesis effects via VEGFR-2 signaling pathway in breast cancer. Breast Cancer Res Treat 134:943–955. https://doi.org/10.1007/s10549-012-1977-9
Yamada T et al (1997) Dietary diosgenin attenuates subacute intestinal inflammation associated with indomethacin in rats. Am J Physiol 273:355–364

Nutraceuticals as Therapeutic Agents for Prevention and Treatment of Diseases

4

Harshita Sachdeva, Sarita Khaturia, and Mamta Chahar

Abstract

Nutraceutical means nutrient and pharmaceutical, also known as medicinal/designer/functional foods as well as phytochemicals. These are the supplements of nutritional value which include daily products like dietary products, genetically modified products, herbal products, bio-yoghurts, vitamins, and fortified cereals. These medicinal or nutraceutical foods are originated from plant sources (garlic, ginger, onion, turmeric), dietary fiber and enzymes like papain and bromelain, various hydrolyzed proteins, and phytonutrients. Traditional nutraceuticals include phytochemicals, probiotic bacteria, enzymes, chemical compounds, nutrients, and plants. Recent investigations have shown that these compounds have promising outcomes in different pathological problems like cancer, diabetes, cardiovascular diseases, and atherosclerosis. These situations involve various changes like redox state alteration, and to counteract this situation, most of the nutraceuticals possess potential toward antioxidant activity. Thus, they are observed as safe sources for promotion of health, mainly to prevent different life-threatening diseases like diabetes, infection, and renal and gastrointestinal disorders, and play a vital role in food and pharmaceutical industries in maintaining life quality, good health, and longevity. Health professionals and nutritionists should research and work together for the benefit of mankind.

H. Sachdeva (✉)
Department of Chemistry, University of Rajasthan, Jaipur, India

S. Khaturia
Department of Chemistry, School of Liberal Arts and Sciences, Mody University of Science and Technology, Laxmangarh, Rajasthan, India
e-mail: saritakhaturia.slas@modyuniversity.ac.in

M. Chahar
Department of Chemistry, Nalanda College of Engineering, Nalanda, Bihar, India

K. K. Behera et al. (eds.), *Prebiotics, Probiotics and Nutraceuticals*,
https://doi.org/10.1007/978-981-16-8990-1_4

Further, allocation of nutraceuticals can be done on the basis of sources of food, their action mechanism, chemical nature, etc. Interest in nutraceuticals is growing rapidly due to the rapid advances in prevention of diseases and healthcare product costs and elderly people using regular food industrial products which allow the enlargement in medicinal premium food products which are marketed to health-conscious people. They offer a great scope, and it is required to execute clinical trials for supplying nutraceuticals which generate huge benefits to customers and service providing companies. Recent trends show that nutraceuticals offer promising results in healthcare sector and nutraceutical consumption is increasing in global market day by day. In the present chapter, much work has been devoted to focus on the recent trends in herbal nutraceuticals for the prevention of cardiovascular disease, cancer, diabetes, hypertension, and arthritis disease.

Keywords

Nutraceuticals · Phytochemicals · Antioxidants · Anticancer · Antidiabetic · CVD · Probiotic

4.1 Introduction About Nutraceuticals

Drugs that are used in the treatment of diseases come in the category of pharmaceuticals and are synthetic compounds. But imagine if we use nutrients for disease prevention and for health promotion. Nutraceuticals are as beneficial as natural foods, while pharmaceutical supplements comprise vitamins and minerals in high concentrations (Bahadoran et al. 2013; Kumar and Kumar 2015). Stephen DeFelice introduced the term “nutraceuticals” in 1989. These nutraceuticals are arranged as substances that are part of our diet and have numerous health benefits as shown in Fig. 4.1 (https://cdn.pixabay.com/photo/2017/09/22/19/05/casserole-dish-2776735__340.jpg). These substances can solve both purposes: nutrition (as they possess nutritional value) and pharmaceutical importance (used in prevention of chronic diseases). Different types of medical problems like cardiovascular diseases, cancer, hypertension, diabetes, and obesity are well treated by using medical benefits of nutraceutical foods. Foods obtained from nature like dietary supplements, different antioxidants, various fortified dairy products such as milk, and natural citrus fruits and vitamins/minerals/herbals/cereals are the common examples of these nutraceuticals (Jadhav et al. 2006; Kalra 2003; Rajasekaran et al. 2008).

They are quite different from pharmaceuticals because instead of isolated vitamins or nutrients they are prepared by conversion of whole foods into a nonfood format. Actually, these nutraceuticals consist of all the health benefits of vegetables. Capsules or tablets that are derived from vegetables like spinach or beet capsules and odorless garlic tablets are popular nutraceuticals (Fig. 4.2) (Navneet et al. 2010). One of the most significant applications of nutraceutical supplements is that they prohibit the disease and promote health benefits by the use of vitamins and minerals found in

Fig. 4.1 Common nutraceuticals (https://cdn.pixabay.com/photo/2017/09/22/19/05/casserole-dish-2776735__340.jpg)

Fig. 4.2 Popular nutraceuticals (https://cdn.pixabay.com/photo/2015/08/25/03/52/alternative-medicine-06144_960_720.jpg)

natural food and must be essential components of our diet (Das et al. 2012a; Navneet et al. 2010).

Due to their outstanding utilization in various fields, they are also known as designer vegetable food, excellent functional food, or potent pharma food. Nutraceuticals are very well utilized to treat various chronic diseases like cancer, diabetes, heart disease, arthritis, osteoporosis, etc. They also possess an important position in clinical therapy because of its low cost and potent activity against the side effects which are generally linked with chemotherapy. With all their potential uses in various fields, nutraceuticals still require more investigation in support to prove their benefits with reduced side effects (Whitman 2001; Heyland 2001). These are used to maintain the individual health and also reduce the risk of different diseases as well as for proper functioning of the body. Further, nutrients act as medicinal foods which are used to maintain health and improve immunity; in this way, they not only prevent but also are used to treat specific diseases. Advantages of nutraceuticals are not only limited to health benefit but also they are well utilized in regular functioning of the body. Literature survey reveals that various nutraceuticals are efficacious to treat different medical conditions (Ramaa et al. 2006).

4.1.1 Sociability of Nutraceuticals

Nutraceuticals are socially available as:

1. Dietary supplements and vitamin products
2. Herbal and botanical products
3. Medical/functional and vitality foods
4. Health/organic/dietetic food
5. Sports/energy products
6. Naturally available medicinal products of various health benefits

4.2 Classification of Nutraceuticals

Sources required for production of nutraceuticals, their pharmacology, and presence of chemical constituents in their framework are the common criteria on which we can classify these magical supplements. According to natural source, all these products can be separated as plant products, animal products, minerals, or microbial products.

4.2.1 Allocation of Nutraceuticals Based on Chemical Groupings

- Nutrients: Nutrients are substances that possess some nutritional value and are used to perform various functions. Nutraceuticals which come in this area are common vitamins/minerals/amino acids/fatty acids, etc. (Fig. 4.3) (https://media.

Fig. 4.3 Nutrients (https://media.istockphoto.com/photos/healthy-eating-selection-of-antioxidant-group-of-food-picture-id1182477420)

Fig. 4.4 Herbs (https://media.istockphoto.com/photos/assortment-of-various-types-of-superfoods-picture-id1173102150)

istockphoto.com/photos/healthy-eating-selection-of-antioxidant-group-of-food-picture-id1182477420).

- Herbals: Common herbs, plant extracts, or various botanical products hold this position (Fig. 4.4) (https://media.istockphoto.com/photos/assortment-of-various-types-of-superfoods-picture-id1173102150).

Fig. 4.5 Dietary supplements (https://cdn.pixabay.com/photo/2018/07/12/00/38/tablets-3532308_960_720.jpg)

- Dietary supplements: Various source-derived reagents like pyruvate/chondroitin sulfate/steroid hormone precursors which are used to perform specific functions like sports nutrition, supplements used for reducing weight and for replacement of meal, and fortified conventional foods, occupy this place (Fig. 4.5) (https://cdn.pixabay.com/photo/2018/07/12/00/38/tablets-3532308_960_720.jpg).

Although these supplements are not connected with the treatment of diseases (Ross 2000), nutraceuticals consider this aspect and played pivotal role in preventing as well as in treating various diseases (Bagchi 2006).

4.2.2 Allocation of Nutraceuticals Based on Food Sources, Their Action Mechanism, and Their Chemical Nature

Dietary Fiber: These are also known as roughage, which are not absorbed by our body and found in whole grains or fruits. Non-starch polysaccharides and some plant components like cellulose, resistant dextrins, lignins, chitins (in fungi), beta-glucans, and oligosaccharides come in this category (Fig. 4.6) (Aportela-Palacios et al. 2005). They can be classified into two types depending on their solubility in water: (1) celluloses/hemicelluloses/lignins are fermented in the colon to a limited extent, and (2) dietary fibers which are soluble include β-glucans/pectins/gums/mucilages/hemicelluloses which are colon fermented.

Probiotics: These can be mistaken as germs responsible for diseases but as we know that our body contains both good and bad types of bacteria. Probiotics include bacteria and yeasts which are good for our digestive system (Fig. 4.7). These are used to keep our gut healthy and working well (Arora et al. 2013). They can be characterized as live microbial feed supplements, which benefit the host animal by

Fig. 4.6 Dietary fiber (https://cdn.pixabay.com/photo/2015/02/27/01/10/grains-651404_960_720.jpg)

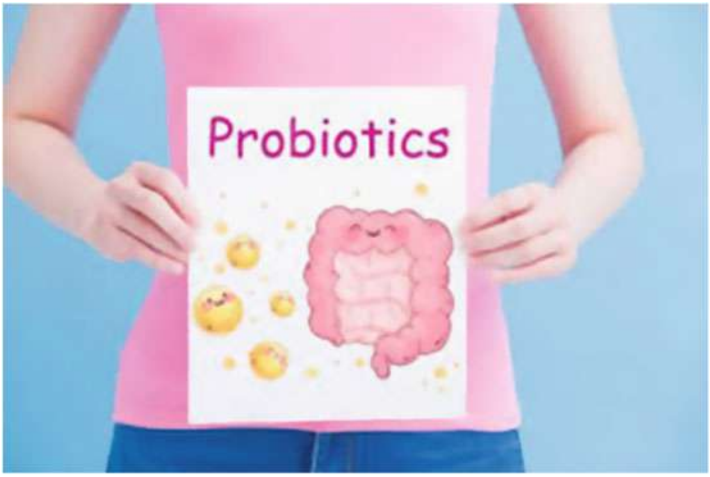


Fig. 4.7 Probiotics (https://depositphotos.com/184196202/stock-photo-woman-probiotics-billboard-blue-background.html)

improving its intestinal microbial balance when administered in appropriate quantities. The following categories of bacteria are included in probiotics:

1. Various species of lactobacilli like *L. acidophilus*/*L. casei*/*L. delbrueckii* subsp. *Bulgaricus*/*L. brevis*/*L. cellobiosus*

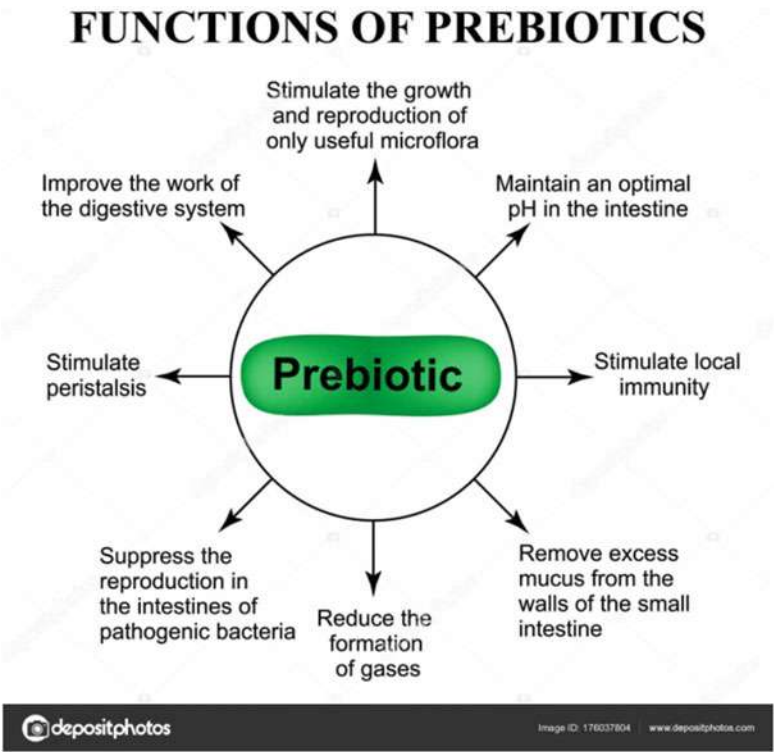


Fig. 4.8 Prebiotics (https://st3.depositphotos.com/3802617/17603/v/1600/depositphotos_176037804-stock-illustration-functions-of-prebiotics-infographics-vector.jpg)

2. Different types of gram-positive cocci like *Lactococcus lactis*/*Streptococcus salivarius* subsp. *thermophilus*/*Enterococcus faecium*
3. Different species of *Bifidobacterium*, e.g., *B. bifidum*/*B. adolescentis*/*B. infantis*, *B. longum*/*B. thermophilum*

Prebiotics: Prebiotics are dietary additives that selectively modify the gut microbiota constitution and metabolism and possess a useful impact on the host. Various fibers that feed the good and friendly bacteria and induce their growth are called prebiotics. Gastrointestinal tract holds very good example in which prebiotics can change an organism's composition in the gut microbiome (Flint et al. 2012) (Fig. 4.8).

PUFA (polyunsaturated fatty acids): These are generally called as "essential fatty acids" and externally added by the diet. They are of two types: omega-3/n-3 and omega-6/n-6 fatty acids which are the superior component of fish oils in comparison to oils of different animals. Omega-3 fatty acids include ALA (α-linolenic acid), EPA (eicosapentaenoic acid), and DHA (docosahexaenoic acid). LA (linoleic acid), GLA (γ-linolenic acid), and ARA (arachidonic acids) are the main omega-6 PUFAs.

Fig. 4.9 Polyunsaturated fatty acids (https://cdn.pixabay.com/photo/2015/08/20/19/18/market-897990_960_720.jpg)

Some of the PUFA like omega-3 fatty acids showed advantages as nutraceuticals and in pharmaceutical fields. The main important function of omega-3 fatty acids is it reduces the risk of diseases related to heart and is also helpful in the development of the retina, brain, and nervous system (Harris et al. 2003) (Fig. 4.9).

Antioxidant vitamins: These are the substances used for protecting the cells from free radicals produced in the process of breaking down of food and exposure to tobacco smoke or radiation. These radicals start damaging the cells and are responsible for heart disease, cancer, and many other diseases (Lobo et al. 2010) (Fig. 4.10).

Polyphenols: A category of compounds that include flavonoids which are a major component of fruits/cereals/beverages and vegetables are known as polyphenols. An important utilization of these micronutrients is that they act as antioxidants which are used for neutralization of free radicals; otherwise, these can start damaging the cells and cause cancer, diabetes, and heart disease (Yao et al. 2004) (Fig. 4.11).

Spices: These are the predominant flavoring and coloring agents used in foods and beverages and become very important nowadays. Spices must possess important nutritional value and antioxidant/antimicrobial and therapeutic properties and are very much effective against different diseases like cancer, cardiovascular diseases, diabetes, and arthritis (Kochhar 2008) (Fig. 4.12).

Food sources of nutraceuticals can be grouped into three categories (Table 4.1) (Johnson et al. 1997). We can also classify nutraceuticals on their mechanism of action and physiological properties (Johnson et al. 1997) (Table 4.2).

Nutraceuticals can also be grouped according to their chemical nature (Zeisel 1999) (Table 4.3).

Fig. 4.10 Antioxidants (https://cdn.pixabay.com/photo/2016/04/21/11/32/groceries-1343141_340.jpg)

Fig. 4.11 Polyphenols (https://cdn.pixabay.com/photo/2017/06/02/19/10/blueberry-2367096_340.jpg)

4.3 Areas Covered by Nutraceuticals

It has been demonstrated that nutraceuticals can help to treat chronic infections or counterbalance harmful side effects as a valuable adjunct to pharmaceuticals. According to Hippocrates' theory, "let the food be thy medicine and thy medicine

Fig. 4.12 Spices (https://cdn.pixabay.com/photo/2016/12/17/18/51/salt-1914130_340.jpg)

Table 4.1 Various nutraceuticals according to food source

Food source	Examples
Plants	Ascorbic acid, gallic acid, β-glucan, cellulose, hemicellulose, glutathione, lutein, luteolin, perillyl alcohol, indole-3-carbinol, daidzein, potassium, geraniol, selenium, lignin, lycopene, minerals
Animals	Cholein, creatine, zinc, docosahexaenoic acid (DHA), conjugated linoleic acid, sphingolipids, calcium, coenzyme Q10
Microbes	*Lactobacillus acidophilus*, *L. acidophilus*, *Streptococcus salivarius*, *Bifidobacterium bifidum*, *B. longum*, *Saccharomyces boulardii* (yeast)

be the food"; it was assumed that human health is related to diet and also there is strong association among acceptable foods required for good health and their valuable use in the treatment of infectious diseases. Food must comply with three basic requirements in order to be considered a "functional food": it must be a part of regular everyday food; it must be obtained from natural sources, taken directly and not in any other pharmacological form; and it must recuperate a particular metabolic process after consumption, thereby avoiding a disease (Trifković and Benković 2019; Santini et al. 2018). Biologically active phytochemicals include alkaloids, terpenoids, and polyphenols, viz., anthocyanins, flavanols, flavones, and isoflavones, and these are the major sources of nutraceutical ingredients. Phytochemicals are nonessential nutrients having disease-preventing properties

Table 4.2 Therapeutic effect of various nutraceuticals

Anticancer	Benefit to lipid profile	Antioxidant activity	Anti-inflammatory	Bone disease
Capsaicin	Beta-glucan	Conjugated linoleic acid	Linolenic acid	Conjugated linoleic acid
Genistein	Gamma-tocotrienol	Beta-carotene	Eicosapentaenoic acid	Soy protein
Daidzein	Delta-tocotrienol	Vitamin C	Docosahexaenoic acid	Genistein
Alpha-tocotrienol	Monounsaturated fatty acids	Polyphenols	Gamma-linolenic acid	Daidzein
Gamma-tocotrienol	Quercetin	Tocopherols	Gamma-linolenic acid	Calcium
L. acidophilus	Resveratrol	Indole-3-carbonol	Quercetin	Fructo-oligosaccharide

Table 4.3 Various nutraceuticals on their chemical nature

Components	Source	Benefits
Isoprenoids		
Carotene, lutein, lycopene	Carrot, tomatoes, fruits, green vegetables	Protect against cancer and heart problems and maintain eye health
Collagen hydrolysate	Gelatin	Improves osteoarthritis
Dietary fiber		
Insoluble fiber	Wheat bran	Reduced risk of colon/breast cancer
β-Glucan	Oats	Lower the risk of CVD
Soluble fiber	Psyllium	Lower the risk of CVD
Flavonoids		
Anthocyanins	Fruits	Protection against metabolic diseases and cancer
Flavonones	Citrus	
Phenols	Fruits, vegetables	Antioxidants, protection against cancer, eye and heart diseases
Caffeic acids	Tuna fish, mineral oils	Lower the risk of CVD, enhance vision
Docosahexaenoic acid	Onion powder, Jerusalem artichokes	Improve gastrointestinal problem
Prebiotics/probiotics		
FOS	Yoghurt, other dairy products	Improve gastrointestinal problem
Lactobacillus		
Soy protein	Soybean	Reduce the risk of heart disease
Thiols		
Diallyl sulfides	Onions, garlics	Lower LDL cholesterol, improve the immune system
Tannins		
Proanthocyanidins	Cocoa, chocolates, cranberries	Maintain the urinary tract, lower the risk of CVD

CVD cardiovascular disease, *FOS* fructo-oligosaccharides which are largely provided by plants. They have unique therapeutic effect on human health and can act as antioxidants, anti-allergic, anti-inflammatory, antibacterial, chemopreventive, antifungal, hepato-protective, hypotensive and neuroprotective agents, and antiaging in osteoporosis, diabetes, genetic mutation, carcinoma, and heart diseases (Calvani et al. 2020). Many of the medicinal areas protected by nutraceuticals include anti-arthritis, respiratory problems like cough and cold, sleep problems, inhibition of growth of cancers and shrinking of tumors, cholesterol regulation, dejection, and analgesics (Das et al. 2012b). Several epidemiological studies indicate that dietary patterns are among the most important factors that determine the occurrence of chronic diseases, like cardiovascular diseases, gall bladder stones, type 2 diabetes (T2D), cataracts, neurodegenerative diseases, and cancer types. Hence, it can be said that human health is directly affected by food and dietary habits (Nivya et al. 2012). The more we use plant-based products, the lesser we will be affected by diseases and infections.

4.3.1 Efficacy of Nutraceuticals Against Cancer

Investigations have shown that a cooperative antioxidant protection mechanism against toxic oxygen intermediates via diet has been recognized for humans. In this context, increased nutritional intake of antioxidants can diminish the threat of diseases such as cancer (Adak and da Silva 2010). Nowadays, it is well-recognized that growth of malignant neoplasm can be reduced by functional foods, nutraceuticals, and additional micronutrients through inhibiting cell multiplication and instigating apoptosis (Calvani et al. 2020), and variety of natural products have demonstrated a possible part in the inhibition and treatment of carcinoma (Zheng et al. 2016).

In most cases, carotenoids arrest the cell cycle associated with the suppression of the expression of cyclin D1, cyclin D2, CDK6, and CDK4 (Bhatt and Patel 2020) or flavonoids inhibiting the growth of cancer cells by modulating the activities of the ROS-scavenging enzyme, engage in cell cycle arrest, induce apoptosis and autophagy, and inhibit the spread and invasiveness of cancer cells (Kopustinskiene et al. 2020). Several reports indicate that phytochemicals can possibly prevent cancer cell growth by suppressing the cancer-linked nuclear transcription factor-κB (NF-κB) pathway and many diseases which cause inflammation (Aparicio-Soto et al. 2019). It has also been reported that RES alters NF-κB activity and it can prevent the CYP1A (cytochrome P450 isoenzyme) metabolism and cyclooxygenase activity. In addition, other metabolism-linked biochemical pathways, oxidation of fatty acids, mitochondrial biogenesis and respiration, and gluconeogenesis may be impaired by RES (Diaz-Gerevini et al. 2016). It has also been suggested to be effective for autoimmune diseases (Han et al. 2015). Curcumin has demonstrated important anticancer effects against different types of cancers, including head and neck cancer, prostate cancer, colorectal cancer, breast cancer, and pancreatic cancer. In addition, its effectiveness and protection in cancer patients have been demonstrated in many clinical trials in human subjects, either alone or in conjunction with other anticancer agents. It is suspected that curcumin exerts its anticancer action through manifold mechanisms by interfering with various cellular paths and by inducing or inhibiting the development of different types of cytokines, enzymes, or growth factors (Tomeh et al. 2019; Das and Vinayak 2015).

The development and progression of breast cancer may be affected by several natural dietary products which include soy, pomegranate, carrot, orange, berries, beetroot, apple, mango, grapes, cruciferous vegetables, ginger, garlic, cereals, etc. Their effects on breast cancer include several modes of action, such as downregulation of expression and activity of ER-α, inhibition of multiplication, movement, metastasis, angiogenesis of tumor cells, apoptosis induction, cell cycle arrest, and radiotherapy and chemotherapy sensitization of breast tumour cells (Li et al. 2017).

4.3.2 Nutraceuticals as Antioxidants

The multistage carcinogenic process includes free radicals (ROS/RNS) which possess unpaired electrons in their valence shell and hence highly reactive, short-lived, and unstable. In the process of achieving stability, it initiates chain reaction and causes damage to living cell inducing cytotoxicity. Cellular DNA oxidative damage may lead to genetic mutations, thereby initiating and progressing multistage transformation of normal cells to cancer cells (Katakwar et al. 2016). The initiation of carcinogenesis can be controlled by antioxidant phytochemicals which prevent DNA damage by using a mechanism composed of enzymatic antioxidants to combat free radicals and neutralize oxidants (Vargas-Mendoza et al. 2019). Similarly, carotenoids and polyphenols can prevent many diseases by reducing OS (Rizzo et al. 2010; Wallace et al. 2016). In recent years, nutraceuticals have received wide attention due to their antioxidant ability (Engwa 2018), and numerous phytochemicals resulting from different plants, herbs, herbal medicines, and spices are capable of activating Nrf2 and inducing in vitro antioxidant or step II detoxifying enzyme expression (Chun et al. 2014). Epidemiological and animal research indicate that eating whole grains, vegetables, and fruits everyday decreases the risk of chronic diseases (Zhang et al. 2015).

4.3.2.1 Phytochemicals

Resveratrol, curcumin, and anthocyanins can reduce inflammation by inhibiting the development of prostaglandin, NF-1B activity, enzyme activity, and increased production of cytokines (Yahfoufi et al. 2018). The chances of death from cardiovascular disease (Yang et al. 2020) tend to be decreased by flavonoids, the widely dispersed phytochemicals in fruits and vegetables.

4.3.2.2 Phenolic Compounds

Flavanol monomers have a number of related compounds consisting of epigallocatechin, epicatechin gallate (EGC), and epigallocatechin gallate (EGCG) (Musial et al. 2020). Carcinogenesis experiments have shown that green tea and

EGCG can prevent the proliferation of cancer cells. Moreover, to inhibit the expression of COX-2 and nitric oxide synthase (NOS), NF-kB activation is blocked by EGCG, curcumin and resveratrol.

4.3.2.3 Berberine

It is another medication which is quite effective against cancer. It is derived from Chinese herbs, capable of binding with oligonucleotides for the stabilization of DNA triplexes or G-quadruplexes, and is capable of reducing the growth of several telomerase and topoisomerase inhibitor tumors (Xiong et al. 2015). Data have shown that in almost all types of cancers, berberine can stop development, carcinogenesis, and metastasis.

4.3.2.4 Lycopene

It is a potent antioxidant, non-provitamin A carotenoid, abundant in tomatoes and their derivatives, and offers defense against free radical cell damage (Milani et al. 2017), thereby preventing cancer (Sahin et al. 2016). Several in vitro studies have shown that lycopene can selectively stop cell growth and, without affecting normal cells, induce apoptosis in cancer cells, and it prevents the development of lung, liver, prostate, colon, and breast tumors as indicated by in vivo studies (Trejo-Solís et al. 2013). Bacanli et al. recently investigated the defensive effects of lycopene against chronic diseases as well as dietary consumption, mechanism of action, bioavailability, and pharmacokinetics of lycopene (Bacanli et al. 2017). Further, it can inhibit the proliferation of cells, interrupt the cell cycle, and cause apoptosis of human breast cancer cells (Takeshima et al. 2014).

4.3.2.5 Curcumin

It shows a wide spectrum of biological activities such as antioxidant, anticancer, antibacterial, antifungal, anti-inflammatory, antiviral, etc. Hence, it is effective against various chronic diseases (Kunnumakkara et al. 2018). It inhibits the proliferation, survival, invasion, angiogenesis, metastasis, chemoresistance, and radiation resistance of cancer cells in different cancer types by modulating various signaling pathways, including NF-κB, and it was proved in approximately over 120 clinical trials that it can treat several chronic conditions without any side effects.

4.3.2.6 Resveratrol (RES)

It is a stilbenoid phenol which can be produced by many plants such as grapes, blueberries, and raspberries with gifted effects on human health. Many studies have shown that it shows very high antioxidant power. It displays antitumor activity and is used to prevent and treat various types of cancers. Furthermore, according to several investigations, resveratrol is capable of inhibiting all phases of carcinogenesis. In addition, it shows other bioactive effects, viz., anti-inflammatory, cardioprotective, vasorelaxant, phytoestrogenic, neuroprotective, etc.(Salehi et al. 2018).

4.3.3 Nutraceuticals in Arthritis

Rheumatoid arthritis is a chronic systemic inflammatory joint condition that mostly affects the pain and weakness of synovial joints (Loseli et al. 2015). Likewise, another chronic, debilitating condition prevalent in our aging population is osteoarthritis (OA). Several antirheumatic drugs are proved to be successful in RA management (Singh et al. 2009). Nonetheless, complementary treatments such as nutraceuticals and supplements are on the rise in joint disease treatment.

4.3.3.1 Glucosamine

It arises in the body naturally and is an aminosaccharide. It is significant for proteoglycan biogenesis, which is crucial for preserving the integrity of the cartilage (Fransen et al. 2014). It is one of the most widely used supplements, with a 1.5 g daily recommended dose, in patients with OA. While it occurs in many forms, the sulfated form of glucosamine is more commonly used in clinical trials. It is deemed healthy, and there have been no previously recorded serious or fatal adverse events. However, since it is derived from chitin found in shellfish (Henrotin et al. 2012), patients with shellfish allergies should use it with caution.

4.3.3.2 Chondroitin

Chondroitin is the main constituent of aggrecan, which forms part of the cartilage (Chou et al. 2005). The bulk of preparations come from cartilaginous extracts from animal sources (Richy et al. 2003). It is maximum sold in sulfate form, like glucosamine. It shows anti-inflammatory, anti-apoptotic, anticatabolic, and antioxidant effect. It is still to be induced straight to human models (Chan et al. 2005; Orth et al. 2002). However, its use was discouraged by the authors as the literature again shows inconsistencies regarding its clinical benefit and that it had minimal or no symptomatic benefits (McAlindon et al. 2014; Reichenbach et al. 2007). A recent study confirmed that chondroitin reduced the decline rate in joint space width at a dose of 800 mg daily (Hochberg 2010).

4.3.3.3 Krill Oil

It results from Antarctic krill (Euphausia superba), phospholipid-rich species and contains a high content of n-3 fatty acids. It is investigated that a mice caused by arthritis with a diet supplemented with krill oil reported significantly lower arthritis ratings and lower joint inflammatory cell infiltration and swelling of the hind paw as compared with control mice (Ierna et al. 2010). Studies suggest that 90 patients get benefitted by using krill oil (Deutsch 2007). However, more studies are required to prove the benefits of krill oil.

4.3.3.4 Fish Oil

It is derived from fatty fish in the body and contains the requisite fatty acids for biological processes. It reduces inflammation and affects metabolic pathways (Caughey et al. 1996). A recent review on the its effect showed substantial improvement in joint tenderness (Cleland et al. 2003). Studies showed that fish oil improved both morning stiffness and tender joint count over a 12-week period (Fortin et al. 1995). A cooperative effect of fish oil and paracetamol was demonstrated by Caughley et al. (Caughey et al. 2010). Adverse reactions reported include diarrhea, intolerance, and gastroesophageal reflux (Fortin et al. 1995). It should be avoided weeks before surgery and there are evidences to support. It may have a poor antiplatelet effect, but recent analyses have reported a no risk of significant postoperative bleeding (Xin et al. 2013).

Other nutraceuticals used in arthritis are gamma-linolenic acid (GLA) (Zurier et al. 1996; Cameron et al. 2011) and avocado-soybean unsaponifiables (ASU) (Maheu et al. 1998; Ernst 2003).

4.3.4 Nutraceuticals in Diabetes Mellitus

Diabetes mellitus is a multifaceted metabolic illness which is related with the development of insulin resistance, reduced insulin signaling and β-cell dysfunction, abnormal glucose and lipid metabolism, subclinical inflammation, and increased oxidative stress. Diet is the main factor which is responsible for the incidence and progress of type 2 diabetes mellitus. A number of investigations show that diet rich in phytochemicals and polyphenolic compounds and with high antioxidant properties are related to lower risk of diabetes (Nimesh and Nimesh 2018; Gray and Threlkeld 2019).

4.3.4.1 Vitamins

An acceptable supply of vitamins may prevent or delay diabetes complications as antioxidants provide protection against oxidative stress (Hathcock 2001; Bartlett and Eperjesi 2008) as indicated by several animal studies. However, there is no clear evidence in humans regarding such investigations (Franzini et al. 2008). Vitamin C is a chain-breaking antioxidant that prevents the propagation of chain reactions and scavenges ROS directly (Riccioni et al. 2007; Chen et al. 2006). Observational studies do not support any link between higher dietary vitamin D consumption and type 2 diabetes occurrences (Abbas et al. 2014). However, an acceptable level of vitamin D has supportive effect on type 2 diabetes prevention (Muñoz-Garach et al. 2019). Low levels of vitamin E are related to increased chances of diabetes, and some studies confirm that people suffering from diabetes have reduced levels of antioxidants. Moreover, it is demonstrated that people with diabetes may also have more antioxidant necessities, due to increased free radical production next to hyperglycemia (Bajaj and Khan 2012).

4.3.4.2 Carbohydrates

The most active substrate having great influence on glycemia levels is carbohydrates, and the key factor responsible for the postprandial response is the whole quantity of carbohydrates, but the type of carbohydrate, cooking method, fiber richness, degree of maturity, etc. can also play a part (Bantle et al. 2008). Most research groups propose that carbohydrate diet should be provided in the form of fruits, pasta, cereals, vegetables, legumes, and tubers (Derosa et al. 2014). Though long-term studies are lacking, it appears that consuming legume starches has an encouraging impact on glycemia as there has been no sudden rise in the persistent effect on postprandial glycemia and may avoid both postprandial hyperglycemia and late hypoglycemia (Russell et al. 2016).

4.3.4.3 Fats

High-fat diets can cause impairment of glucose tolerance and promote obesity, dyslipidemia (abnormal levels of cholesterol), and atherosclerotic heart disease as indicated by numerous studies (Gao et al. 2014). These abnormalities can be improved by dropping saturated fat intake as showed by research results (Siri-Tarino et al. 2010). Research suggests that canola and peanut and olive oils may have valuable effects on triglycerides and glycemic control in some diabetes patients (Mozaffarian 2016; Sievenpiper et al. 2018).

4.3.4.4 Fiber

High intake of dietary fiber is related with lower mortality rate in people with diabetes (Burger et al. 2012). Patients with diabetes should consume 20 to 35 g of fiber from raw vegetables and unprocessed grains per day (American Diabetes Association 2019). There is no need to raise the dose of fiber in diabetic patients (Higgins 2004).

4.3.4.5 Chromium

Diabetic patients may be deficient in trace element chromium (Lau et al. 2008; Costello et al. 2016). It is suggested in patients with type 2 diabetes mellitus that chromium supplements can improve glucose acceptance. But there is no clear evidence for the benefit of chromium supplement in diabetic patients (American Diabetes Association 2007).

4.3.4.6 Magnesium

It is well established that magnesium-rich diets are good for diabetic patients. Magnesium deficiency inhibits cellular defenses against oxidation damage, resulting in decreased resilience to diabetes-induced oxidative stress, thereby accelerating the progression of diabetes-related complications (Dubey et al. 2020; Dibaba et al. 2014), and hypomagnesemia may exacerbate T2DM. Animal studies showed that administration of dietary Mg (50 mg/mL of drinking water) causes reduction in glucose levels in blood, increase in mitochondrial function, and decrease in oxidative stress in mice with diabetes for 6 weeks (Liu et al. 2019).

Other nutraceuticals which play important role in diabetes are *lipoic acid* which is naturally occurring antioxidant having strong ROS-scavenging activity (Ziegler et al. 1999). *Vanadium* is investigated as probable beneficial agent for the treatment of diabetes. Recently, Trevino et al. reported the medicinal property of vanadate- and polioxovanadate-containing compounds in the treatment of diabetes mellitus (Treviño et al. 2019; Snow 2006), *protein* (Canadian Minister of National Health and Welfare Nutrition Recommendations 1990; Nuttall and Gannon 1991; Kontessis et al. 1990; Patel 2015), *coenzyme Q10* (Singh et al. 1999), and *L-carnitine* (Rajasekar and Anuradha 2007; Calo et al. 2006).

4.4 Future Aspects of Nutraceuticals

Transformation in eating habits, less physical work, work stress, more desk jobs, and long working hours have made people more vulnerable to lifestyle diseases. Lack of timing and nutritious food has led to the rise in nutritional supplements. Nowadays' increase awareness in people on health problems and their urge to take preventive steps have been driving the nutraceuticals industry. People are going toward healthier lifestyle, exercise more, and eat healthy food; this development expands the nutraceutical markets globally. Nutraceutical study is changing constantly which reflects research, innovation, and consumer demand. By looking at the present impact of nutraceuticals and its growing importance in the international market, we can predict its leading future. Vitamins and minerals are common supplements used as food intake. These dietary supplements provide health benefit. Additionally, awareness for diet and nutrition is significantly growing in recent years and dietary supplement industries are further supported by governments desperate to reduce their burden on healthcare systems.

The demand of nutraceuticals will increase in the future along with the following aspects:

1. Functional food
   Functional foods are food or food ingredient that has some specific health benefits which are beyond the typical nutrients. Now, people are more health-conscious; therefore, the demand of nutritional food will increase in the future.
2. New entries
   The advancements in the arena of food supplements that are enriched in microorganisms including probiotics, prebiotics, and symbiotic bacterium.
3. Utilization of analytical devices
   For the analysis of composition of plant extracts, various analysis devices are used for the characterization of chemical structures. TL and HPTLC are used for the analytical separation. More advancements are required for developing new devices.
4. Medicines
   Oral aloe sterol enhances the formation of type I and type III collagen I human dermal fibroblasts which improve the skin elasticity (Tanaka et al. 2015) derived from *Aloe vera* gel. *Serenoa repens* is an herbal treatment for prostatic and urinary diseases (Agbabiaka et al. 2009).

4.5 Conclusions

In recent years, unhealthy eating habits and fast-changing lifestyle have led to increase diseases like hypertension, diabetes, cancer, etc. The increase in awareness and interest of consumer about the benefits of nutrition and long term diet has contributed to a favorable market for nutraceutical industry. From decades, research is focused on several nutraceuticals such as PUFA or vitamins which might stimulate the defense systems of our body. The globe is not far where nutraceuticals will be our preferred prescription of tomorrow.

References

Abbas S, Linseisen J, Rohrmann S, Beulens JW, Buijsse B, Amiano P et al (2014) Dietary vitamin D intake and risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition: the EPIC-InterAct study. Eur J Clin Nutr 68(2):196–202

Adak M, da Silva JA (2010) Garlic [Allium sativum] and its beneficial effect on cardiovascular disease: a review. Int J Biomed Pharm Sci 4(1):1–20

Agbabiaka TB, Pittler MH, Wider B, Ernst E (2009) Serenoarepens: a systematic review of adverse events. Drug Saf 32(8):637–647

American Diabetes Association (2007) Nutrition recommendations and interventions for diabetes (position statement). Diabetes Care 30(Suppl 1):S48–S65

American Diabetes Association (2019) Diabetes Care 42(Supplement 1):S46–S60

Aparicio-Soto M, Redhu D, Sánchez-Hidalgo M, Fernández-Bolaños JG, Alarcón-de-la-Lastra C et al (2019) Olive-oil-derived polyphenols effectively attenuate inflammatory responses of human keratinocytes by interfering with the NF-κB pathway. Mol Nutr Food Res 63(21): 1900019

Aportela-Palacios A, Sosa-Morales ME, Vélez-Ruiz JF (2005) Rheological and physicochemical behavior of fortified yogurt, with fiber and calcium. J Texture Stud 36(3):333–349

Arora M, Sharma S, Baldi A (2013) Comparative insight of regulatory guidelines for probiotics in USA, India and Malaysia: a critical review. Int J Biotechnol Wellness Ind 2(2):51–64

Bacanli M, Başaran N, Başaran AA (2017) Lycopene: is it beneficial to human health as an antioxidant? Turk J Pharm Sci 14(3):311

Bagchi D (2006) Nutraceuticals and functional foods regulations in the United States and around the world. Toxicology 221(1):1–3

Bahadoran Z, Mirmiran P, Azizi F (2013) Dietary polyphenols as potential nutraceuticals in management of diabetes: a review. J Diabetes Metab Disord 12(1):43

Bajaj S, Khan A (2012) Antioxidants and diabetes. Indian J Endocrinol Metabol 16(Suppl 2):267

Bantle JP, Wylie-Rosett J, Albright AL et al (2008) Nutrition recommendations and interventions for diabetes: a position statement of the American Diabetes Association. Diabetes Care 31 (Suppl 1):S61–S78

Bartlett HE, Eperjesi F (2008) Nutritional supplementation for type 2 diabetes: A systematic review. Ophthalmic Physiol Opt 28(6):503–523

Bhatt T, Patel K (2020) Carotenoids: potent to prevent diseases review. Nat Prod Bioprospect 10(3): 109

Burger KNJ, Beulens JWJ, van der Schouw YT, Sluijs I, Spijkerman AMW, Sluik D et al (2012) Dietary fiber, carbohydrate quality and quantity, and mortality risk of individuals with diabetes mellitus. PLoS ONE 7(8):e43127

Calo LA, Pagnin E, Davis PA et al (2006) Antioxidant effect of L-carnitine and its short chain esters: relevance for the protection from oxidative stress related cardiovascular damage. Int J Cardiol 107(1):54–60

Calvani M, Pasha A, Favre C (2020) Nutraceutical boom in cancer: inside the labyrinth of reactive oxygen species. Int J Mol Sci 21(6):1936

Cameron M, Gagnier JJ, Chrubasik S (2011) Herbal therapy for treating rheumatoid arthritis. Cochrane Database Syst Rev 8:CD002948

Canadian Minister of National Health and Welfare Nutrition Recommendations (1990) The report of the scientific review committee. Canadian Government Publishing Centre, Ottawa

Caughey GE, Mantzioris E, Gibson RA, Cleland LG, James MJ (1996) The effect on human tumor necrosis factor alpha and interleukin 1 beta production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. Am J Clin Nutr 63(1):116–122
Caughey GE, James MJ, Proudman SM, Cleland LG (2010) Fish oil supplementation increases the cyclooxygenase inhibitory activity of paracetamol in rheumatoid arthritis patients. Complement Ther Med 18(3-4):171–174
Chan PS, Caron JP, Orth MW (2005) Effect of glucosamine and chondroitin sulfate on regulation of gene expression of proteolytic enzymes and their inhibitors in interleukin-1-challenged bovine articular cartilage explants. Am J Vet Res 66:1870–1876
Chen H, Karne RJ, Hall G et al (2006) High-dose oral vitamin C partially replenishes vitamin C levels in patients with type 2 diabetes and low vitamin C levels but does not improve endothelial dysfunction or insulin resistance. Am J Physiol Heart Circ Physiol 290(1):137–145
Chou MM, Vergnolle N, McDougall JJ, Wallace JL, Marty S et al (2005) Effects of chondroitin and glucosamine sulfate in a dietary bar formulation on inflammation, interleukin-1beta, matrix metalloprotease-9, and cartilage damage in arthritis. Exp Biol Med 230:255–262
Chun KS, Kundu J, Kundu JK, Surh YJ (2014) Targeting Nrf2-Keap1 signaling for chemoprevention of skin carcinogenesis with bioactive phytochemicals. Toxicol Lett 229(1):73–84
Cleland LG, James MJ, Proudman SM (2003) The role of fish oils in the treatment of rheumatoid arthritis. Drugs 63(9):845–853
Costello RB, Dwyer JT, Bailey RL (2016) Chromium supplements for glycemic control in type 2 diabetes: limited evidence of effectiveness. Nutr Rev 74(7):455–468
Das L, Vinayak M (2015) Long term effect of curcumin in restoration of tumour suppressor p53 and phase-II antioxidant enzymes via activation of NRF2 signalling and modulation of inflammation in prevention of cancer. PLoS ONE 10:e0124000
Das L, Bhaumik E, Raychaudhuri U, Chakraborty R (2012a) Role of nutraceuticals in human health. J Food Sci Technol 49(2):173–183
Das L, Bhaumik E, Raychaudhuri U, Chakraborty R (2012b) Role of nutraceuticals in human health. J Food Sci Technol 49(2):173–183
Derosa G, Limas CP, Macías PC, Estrella A, Maffioli P (2014) Dietary and nutraceutical approach to type 2 diabetes. Arch Med Sci 10(2):336
Deutsch L (2007) Evaluation of the effect of Neptune krill oil on chronic inflammation and arthritic symptoms. J Am Coll Nutr 26(1):39–48
Diaz-Gerevini GT, Repossi G, Dain A, Tarres MC, Das UN, Eynard AR (2016) Beneficial action of resveratrol: how and why? Nutrition 32(2):174–178
Dibaba DT, Xun P, Fly AD, Yokota K, He K (2014) Dietary magnesium intake and risk of metabolic syndrome: a meta-analysis. Diabet Med 31(11):1301–1309
Dubey P, Thakur V, Chattopadhyay M (2020) Role of minerals and trace elements in diabetes and insulin resistance. Nutrients 12(6):1864
Engwa GA (2018) Free radicals and the role of plant phytochemicals as antioxidants against oxidative stress-related diseases. Phytochemicals 2018:49–73
Ernst E (2003) Avocado–soybean unsaponifiables (ASU) for osteoarthritis–a systematic review. Clin Rheumatol 22(4-5):285–288
Flint HJ, Scott KP, Louis P, Duncan SH (2012) The role of the gut microbiota in nutrition and health. Nat Rev Gastroenterol Hepatol 9(10):577
Fortin PR, Lew RA, Liang MH, Wright EA, Beckett LA et al (1995) Validation of a meta-analysis: the effects of fish oil in rheumatoid arthritis. J Clin Epidemiol 48(11):1379–1390
Fransen M, Agaliotis M, Nairn L, Votrubec M, Bridgett L et al (2014) Glucosamine and chondroitin for knee osteoarthritis: A double-blind randomised placebo-controlled clinical trial evaluating single and combination regimens. Ann Rheum 74:851–858
Franzini L, Ardigo D, Zavaroni I (2008) Dietary antioxidants and glucose metabolism. Curr Opin Clin Nutr Metab Care 11(4):471–476
Gao X, Liu X, Xu J, Xue C, Xue Y, Wang Y (2014) Dietary trimethylamine N-oxide exacerbates impaired glucose tolerance in mice fed a high fat diet. J Biosci Bioeng 118(4):476–481

Gray A, Threlkeld RJ (2019) Nutritional recommendations for individuals with diabetes. In: Endotext. MDText.com, Inc., South Dartmouth

Han G, Xia J, Gao J, Inagaki Y, Tang W, Kokudo N (2015) Anti-tumor effects and cellular mechanisms of resveratrol. Drug Discoveries Therap 9(1):1–2

Harris WS, Park Y, Isley WL (2003) Cardiovascular disease and long-chain omega-3 fatty acids. Curr Opin Lipidol 14:9–14

Hathcock J (2001) Dietary supplement: how they are used and regulated. J Nutr 131(3):1114–1117

Henrotin Y, Mobasheri A, Marty M (2012) Is there any scientific evidence for the use of glucosamine in the management of human osteoarthritis? Arthritis Res Ther 14:201

Heyland DK (2001) In search of the magic nutraceutical: problems with current approaches. J Nutr 131(9):2591

Higgins JA (2004) Resistant starch: metabolic effects and potential health benefits. J AOAC Int 87(3):761–768

Hochberg MC (2010) Structure-modifying effects of chondroitin sulfate in knee osteoarthritis: an updated meta-analysis of randomized placebo-controlled trials of 2-year duration. Osteoarthr Cartil 18:28–31

Ierna M, Kerr A, Scales H, Berge K, Griinari M (2010) Supplementation of diet with krill oil protects against experimental rheumatoid arthritis. BMC Musculoskelet Disord 11(1):136

Jadhav D, Deshpande D, Ramaa CS, Kadam VJ (2006) Nutraceuticals and functional foods: a new era in health and disease management. Indian J Pharm Educ Res 40(3):190

Johnson MM, Swan DD, Surette ME, Stegner J, Chilton T, Fonteh AN, Chilton FH (1997) Dietary supplementation with γ-linolenic acid alters fatty acid content and eicosanoid production in healthy humans. J Nutr 127(8):1435–1444

Kalra EK (2003) Nutraceutical-definition and introduction. AAPS PharmSci 5(3):27–28

Katakwar P, Metgud R, Naik S, Mittal R (2016) Oxidative stress marker in oral cancer: a review. J Cancer Res Ther 12(2):438

Kochhar KP (2008) Dietary spices in health and diseases: I. Indian J Physiol Pharmacol 52(2): 106–122

Kontessis P, Jones S, Dodds R et al (1990) Renal, metabolic and hormonal responses to ingestion of animal and vegetable proteins. Kidney Int 38(1):136–144

Kopustinskiene DM, Jakstas V, Savickas A, Bernatoniene J (2020) Flavonoids as anticancer agents. Nutrients 12(2):457

Kumar K, Kumar S (2015) Role of nutraceuticals in health and disease prevention: a review. South Asian J Food Technol Environ 1:116–121

Kunnumakkara AB, Sailo BL, Banik K, Harsha C, Prasad S et al (2018) Chronic diseases, inflammation, and spices: how are they linked? J Transl Med 16(1):14

Lau FC, Bagchi M, Sen CK et al (2008) Nutrigenomic basis of beneficial effects of chromium (III) on obesity and diabetes. Mol Cell Biochem 317(1-2):1–10

Li Y, Li S, Meng X, Gan RY, Zhang JJ, Li HB (2017) Dietary natural products for prevention and treatment of breast cancer. Nutrients 9(7):728

Liu M, Jeong EM, Liu H, Xie A, So EY et al (2019) Magnesium supplementation improves diabetic mitochondrial and cardiac diastolic function. JCI Insight 4(1):e123182

Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: impact on human health. Pharmacogn Rev 4(8):118

Loseli M, Awwad GE, Bradshaw AR (2015) A review of nutraceuticals in joint arthritis. J Pain Relief 4:180

Maheu E, Mazières B, Valat JP, Loyau G, Loët XL et al (1998) Symptomatic efficacy of avocado/ soybean unsaponifiables in the treatment of osteoarthritis of the knee and hip: a prospective, randomized, double-blind, placebo-controlled, multicenter clinical trial with a six-month treatment period and a two-month follow-up demonstrating a persistent effect. Arthritis Rheum 41(1):81–91

McAlindon TE, Bannuru R, Sullivan MC, Arden NK, Berenbaum F et al (2014) OARSI guidelines for the non-surgical management of knee osteoarthritis. Osteoarthr Cartil 22(3):363–388

Milani A, Basirnejad M, Shahbazi S, Bolhassani A (2017) Carotenoids: biochemistry, pharmacology and treatment. Br J Pharmacol 174(11):1290–1324
Mozaffarian D (2016) Dietary and policy priorities for cardiovascular disease, diabetes, and obesity: a comprehensive review. Circulation 133(2):187–225
Muñoz-Garach A, García-Fontana B, Muñoz-Torres M (2019) Vitamin D status, calcium intake and risk of developing type 2 diabetes: an unresolved issue. Nutrients 11(3):642
Musial C, Kuban-Jankowska A, Gorska-Ponikowska M (2020) Beneficial properties of green tea catechins. Int J Mol Sci 21(5):1744
Navneet T, Gupta BP, Nagariya AK, Jain NP, Jitendra B, Surendra J (2010) Nutraceutical: new era's safe pharmaceuticals. J Pharm Res 3(6):1243–1247
Nimesh S, Nimesh VD (2018) Nutraceuticals in the management of diabetes mellitus. Pharm Pharmacol Int J 6(2):114–120
Nivya MA, Kaliyappan R, Vel K, Sasidharan S, Seethpathy GS (2012) Role of nutraceutical in cancer. Int J Pharm Pharm Sci 4:14–17
Nuttall FQ, Gannon MC (1991) Plasma glucose and insulin response to macronutrients in nondiabetic and NIDDM subjects. Diabetes Care 14(9):824–838
Orth MW, Peters TL, Hawkins JN (2002) Inhibition of articular cartilage degradation by glucosamine-HCl and chondroitin sulphate. Equine Vet J 34(S34):224–229
Patel S (2015) Emerging trends in nutraceutical applications of whey protein and its derivatives. J Food Sci Technol 52(11):6847–6858
Rajasekar P, Anuradha CV (2007) Effect of L-carnitine on skeletal muscle lipids and oxidative stress in rats fed high fructose diet. Exp Diabetes Res 2007:72741
Rajasekaran A, Sivagnanam G, Xavier R (2008) Nutraceuticals as therapeutic agents: a review. Res J Pharm Technol 1(4):328–340
Ramaa CS, Shirode AR, Mundada AS, Kadam VJ (2006) Nutraceuticals-an emerging era in the treatment and prevention of cardiovascular diseases. Curr Pharm Biotechnol 7(1):15–23
Reichenbach S, Sterchi R, Scherer M, Trelle S, Bürgi E et al (2007) Meta-analysis: chondroitin for osteoarthritis of the knee or hip. Ann Intern Med 146(8):580–590
Riccioni G, Bucciarelli T, Mancini B et al (2007) Antioxidant vitamin supplementation in cardiovascular diseases. Ann Clin Lab Sci 37(1):89–95
Richy F, Bruyere O, Ethgen O, Cucherat M, Henrotin Y et al (2003) Structural and symptomatic efficacy of glucosamine and chondroitin in knee osteoarthritis: a comprehensive meta-analysis. Arch Intern Med 163:1514–1522
Rizzo AM, Berselli P, Zava S, Montorfano G, Negroni M et al (2010) Endogenous antioxidants and radical scavengers. In: Bio-farms for nutraceuticals. Springer, Boston, MA, pp 52–67
Ross S (2000) Functional foods: the food and drug administration perspectives. Am J Clin Nutr 71(6):1735
Russell WR, Baka A, Björck I, Delzenne N, Gao D, Griffiths HR et al (2016) Impact of diet composition on blood glucose regulation. Crit Rev Food Sci Nutr 56(4):541–590
Sahin K, Ali S, Sahin N, Orhan C, Kucuk O (2016) Lycopene: multitargeted applications in cancer therapy. Nat Prod Cancer Drug Discovery 2016:79–108
Salehi B, Mishra AP, Nigam M, Sener B, Kilic M, Sharifi-Rad M, Fokou PV, Martins N, Sharifi-Rad J (2018) Resveratrol: a double-edged sword in health benefits. Biomedicine 6(3):91
Santini A, Cammarata SM, Capone G, Ianaro A, Tenore GC et al (2018) Nutraceuticals: opening the debate or a regulatory framework. Br J Clin Pharmacol 84(4):659–672
Sievenpiper JL, Chan CB, Dworatzek PD, Freeze C, Williams SL (2018) Nutrition therapy. Can J Diabetes 42:64–79
Singh RB, Niaz MA, Rastogi SS et al (1999) Effect of hydro soluble coenzyme Q10 on blood pressures and insulin resistance in hypertensive patients with coronary artery disease. J Hum Hypertens 13(3):203–208
Singh JA, Christensen R, Wells GA, Suarez-Almazor ME, Buchbinder R et al (2009) Biologics for rheumatoid arthritis: an overview of Cochrane reviews. Cochrane Database Syst Rev 4: CD007848

Siri-Tarino PW, Sun Q, Hu FB, Krauss RM (2010) Saturated fatty acids and risk of coronary heart disease: modulation by replacement nutrients. Curr Atheroscler Rep 12(6):384–390

Snow W (2006) Managing editor with diabetes. Surging some look for alternative treatment

Takeshima M, Ono M, Higuchi T, Chen C, Hara T, Nakano S (2014) Anti-Proliferative and apoptosis-inducing activity of lycopene against three subtypes of human breast cancer cell lines. Cancer Sci 105:252–257

Tanaka M, Misawa E, Yamauchi K, Abe F, Ishizaki C (2015) Effects of plant sterols derived from Aloe vera gel on human dermal fibroblasts in vitro and on skin condition in Japanese women. Clin Cosmet Investig Dermatol 8:95

Tomeh MA, Hadianamrei R, Zhao X (2019) A review of curcumin and its derivatives as anticancer agents. Int J Mol Sci 20(5):1033

Trejo-Solís C, Pedraza-Chaverrí J, Torres-Ramos M, Jiménez-Farfán D, Cruz Salgado A et al (2013) Multiple molecular and cellular mechanisms of action of lycopene in cancer inhibition. Evid Based Complement Alternat Med 2013:705121

Treviño S, Díaz A, Sánchez-Lara E, Sanchez-Gaytan BL, Perez-Aguilar JM et al (2019) Vanadium in biological action: chemical, pharmacological aspects, and metabolic implications in diabetes mellitus. Biol Trace Elem Res 188(1):68–98

Trifković K, Benković M (2019) Introduction to nutraceuticals and pharmaceuticals. In: Nutraceuticals and natural product pharmaceuticals. Academic Press, Cambridge, pp 1–31

Vargas-Mendoza N, Morales-González Á, Madrigal-Santillán EO, Madrigal-Bujaidar E, Álvarez-González I et al (2019) Antioxidant and adaptative response mediated by Nrf2 during physical exercise. Antioxidants 8(6):196

Wallace TC, Slavin M, Frankenfeld CL (2016) Systematic review of anthocyanins and markers of cardiovascular disease. Nutrients 8(1):32

Whitman MM (2001) Understanding the perceived need for complementary and alternative nutraceuticals: lifestyle issues. Clin J Oncol Nurs 5:5

Xin W, Wei W, Lin Z, Zhang X, Yang H et al (2013) Fish oil and atrial fibrillation after cardiac surgery: a meta-analysis of randomized controlled trials. PLoS One 8(9):e72913

Xiong YX, Su HF, Lv P, Ma Y, Wang SK, Miao H et al (2015) A newly identified berberine derivative induces cancer cell senescence by stabilizing endogenous G-quadruplexes and sparking a DNA damage response at the telomere region. Oncotarget 6(34):35625

Yahfoufi N, Alsadi N, Jambi M, Matar C (2018) The immunomodulatory and anti-inflammatory role of polyphenols. Nutrients 10(11):1618

Yang Y, Dong JY, Cui R, Muraki I, Yamagishi K et al (2020) Japan Public Health Center-based Prospective Study Group Consumption of flavonoid-rich fruits and risk of coronary heart disease: a prospective cohort study. Br J Nutr 9:1–26

Yao LH, Jiang YM, Shi J, Tomas-Barberan FA, Datta N et al (2004) Flavonoids in food and their health benefits. Plant Foods Hum Nutr 59(3):113–122

Zeisel SH (1999) Regulation of nutraceuticals. Science 285:185–186

Zhang YJ, Gan RY, Li S, Zhou Y, Li AN et al (2015) Antioxidant phytochemicals for the prevention and treatment of chronic diseases. Molecules 20(12):21138–21156

Zheng J, Zhou Y, Li Y, Xu DP, Li S, Li HB (2016) Spices for prevention and treatment of cancers. Nutrients 8:495

Ziegler D, Reljanovic M, Mehnert H et al (1999) Alpha-lipoic acid in the treatment of diabetic polyneuropathy in Germany: current evidence from clinical trials. Exp Clin Endocrinol Diabetes 107(7):421–430

Zurier RB, Rossetti RG, Jacobson EW, Demarco DM, Liu NY et al (1996) Gamma-linolenic acid treatment of rheumatoid arthritis A randomized, placebo-controlled trial. Arthritis Rheum 39(11):1808–1817

Probiotics as Efficacious Therapeutic Option for Treating Gut-Related Diseases: Molecular and Immunobiological Perspectives

5

Nabarun Chandra Das, Ritwik Patra, Atrayee Dey, and Suprabhat Mukherjee

Abstract

Current trends of food habits to lead a modern lifestyle with inadequate sleep have become one vital aspect to make our gut unhealthy. Infective gut with inflamed ulcer has now become an overgrowing concern to the whole world. The human gut coevolved with several microorganisms that maintain a symbiotic relationship and influence the gut to sustain a proper shape and function. Thus, dysbiosis or misbalancing in gut microbiome leads to various health issues that not only are restricted to the gut such as inflammatory bowel disease, cancer, diarrhea, and allergy but also affect the heart, kidney, and liver. Therefore, to overcome such life-threatening outcomes, a therapeutic solution is needed to select and have a sufficient potential in rebuilding eubiosis in the gut. In this concerned period, probiotics can be a promising prophylaxis as several studies explored its capabilities as life savior from such diseases. Herein, we emphasize the role of probiotics in treating gut-related inflammatory and infectious diseases on a molecular and cellular basis.

Keywords

Probiotics · Gut microbes · Inflammatory disease · Infectious disease · Cytokines

N. C. Das · R. Patra · S. Mukherjee (✉)
Integrative Biochemistry and Immunology Laboratory, Department of Animal Science, Kazi Nazrul University, Asansol, West Bengal, India
e-mail: suprabhat.mukherjee@knu.ac.in

A. Dey
Postgraduate Department of Zoology, Banwarilal Bhalotia College, Asansol, West Bengal, India

K. K. Behera et al. (eds.), *Prebiotics, Probiotics and Nutraceuticals*,
https://doi.org/10.1007/978-981-16-8990-1_5

5.1 Introduction

Microflora or microbiota means the assemblage of microorganisms such as bacteria, archaea, fungi, protozoans, and viruses that inhabit in all the peripheries of the mammalian gastrointestinal tract (Sekirov et al. 2010). The gastrointestinal tract (GI) of an adult human inhabits approximately 100 trillion microorganisms (Backhed et al. 2005). Gut microflora are generally beneficial to the host (Holzapfel et al. 2001) because they are capable of fermenting dietary fiber into short-chain fatty acid which is absorbed by the host. Some others synthesize vitamin K that cannot be synthesized by the host. Besides, these gut microflorae provide various positive effects on the host like helping strengthening the integrity of the gut, maintaining proper shape of the intestinal epithelium (Natividad and Verdu 2013), inhibiting invasion of pathogen (Bäumler and Sperandio 2016), harvesting energy (Den Besten et al. 2013), and boosting the immunity of the host (Gensollen et al. 2016). In order to defend injuries and preserve homeostasis, the GI tract restricts exposure of the host immune system to the microbiota by incorporating multifactorial and dynamic intestinal barriers (Thursby and Juge 2017). Hence, it can be said that there is immediate symbiotic relationship between the gut microbiota and host. Shaping of gut microflora depends on several environmental factors like geographical location or isolation, surgery, smoking, mental stress, and lifestyle in both urban and rural areas. Treatments with antibiotic dramatically disrupt microbial balance and also the richness and community diversity. Clindamycin, metronidazole, and ciprofloxacin have all been reported to affect the structure of microbiota in due course of time (Jernberg et al. 2007; Jakobsson et al. 2010; Dethlefsen and Relman 2011). Microbiomes serve permanent considerable functions by accelerating digestion of food, promoting metabolism of xenobiotics, and controlling of innate and adaptive immunological processes. Intense cross-talk between host and gut bacteria maintains proper homeostasis. Various factors like food habit, age, depression, and illness result in elevated or decremented relative abundance and diversity of bacterial specie in the GI and other body sites (Belizário and Faintuch 2018). In comparison to cells in the body, there are more bacteria in the gastrointestinal tract; therefore, it is vital to maintain a balance between good bacteria and bad bacteria. When bacterial imbalance occurs in the digestive tract, it is termed as dysbiosis. The type of diet has the potential role in determining bowel bacterial balance because different species of bacteria thrive on different types of carbohydrates, fiber, fat, and protein. Dysbiosis develops when the number of probiotic bacteria decreases and other bad bacteria become overly abundant. Many studies reported that continuous disruption of gut microbial community, i.e., dysbiosis, relates to inflammatory bowel diseases (IBD), irritable bowel syndrome (IBS), and multiple diseases such as diabetes, excessive weight gain or obesity, cancer, and cardiovascular and central nervous system disorders (Belizário and Faintuch 2018). The onset of dysbiosis can be prevented by the application of probiotics. Probiotic-rich food can restore the proper rhythm of beneficial and pathogenic bacteria in the digestive tract.

Therapeutic usage of probiotics has been carried out for many years in diverse places of the world for their significant role in enhancing the longevity of digestive health (Cani 2018). According to the World Health Organization (WHO), the definition of probiotic may be concluded as "live organisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO 2001). In the present day, taking probiotic strains as food supplements can be recommended as a precautionary approach to maintain the balance of the intestinal microflora and also remediate the wellness of humans as well as animals (Holzapfel et al. 2001). Many investigations reported that probiotics can exert widespread therapeutic effects that involve modulation of immunity, elevating the level of serum cholesterol, treating atherosclerosis and arteriosclerosis, treating rheumatoid arthritis, preventing cancer, improving lactose intolerance, and preventing or reducing the effects of atopic dermatitis and diarrhea.

Another disease known as inflammatory bowel disease (IBD) (Vanderpool et al. 2008) refers to the existence of a group of pathogenic microflorae characterized by inflammation of the small intestine and colon (Fernández et al. 2019). Crohn's disease and ulcerative colitis have been observed in developing countries throughout the recent times and are caused by accumulation of genetic and environmental factors, disruption of the intestinal epithelium, and dysbiosis of the gut microbiome (Barra et al. 2020). Therefore, development in the use of probiotics has helped us in the management and production of intestinal biosensors that serve as diagnostic tools.

Generally, an immune response is initiated by innate immunity when exposed to foreign substances (antigen) or tissue injury. Innate immunity helps to prime adaptive immune responses against persistent inflammation. Various beneficial effects of probiotics on the intestinal mucosal of the host have been recognized. Pathogenic effects of harmful bacteria are inhibited by probiotics (sometimes indigenous microbiota) by releasing bactericidal substances and competing with pathogens for attachment to the intestinal epithelium.

Probiotics have been observed to elevate the innate immunity and modulate pathogen-induced inflammation with the help of toll-like receptor-regulated signaling pathways (Taverniti and Guglielmetti 2011). The purpose of this chapter is to address the role of probiotics in therapeutic areas for treating gut-related inflammatory and infectious diseases with molecular and immunological perspectives.

5.2 Microbiology of Probiotics

5.2.1 Taxonomy

Probiotics are a variety of different microorganisms that confer health benefits. The genera *Lactobacillus* is composed of 261 species of bacteria that are named based on their unique characteristics (Zheng et al. 2020). Conventionally, classification of lactic acid bacteria is done on the basis of phenotypic characteristics like structural organization, method of glucose fermentation, fermentation of various carbohydrates, growth pattern at various temperature, and lactic acid arrangement. On the other hand, investigations on the basis of correlative 16S ribosomal RNA sequencing studies revealed that some taxa developed in accordance with structural organizations do not correlate with the suggested phylogenetic relations. Therefore, some species cannot be immediately differentiated by phenotypic features. Contemporary molecular techniques such as PCR (polymerase chain reaction) and other various genotypic methodologies are becoming gradually essential for identification of species and distinguishing probiotic strain. Different types of molecular typing methodologies such as pulsed-field gel electrophoresis, repetitive polymerase chain reaction, and restriction fragment length polymorphism are exclusively essential for distinct portrayal and observation of such strains chosen for implementation as probiotics (Zhang et al. 2016). Table 5.1 encloses a list of probiotics available in the market.

5.2.2 Biochemical Characteristics

Probiotics help to set microbial equilibrium in the gastrointestinal tract. Lactic acid bacteria (LAB) are familiar probiotic entity and protective cultures that are considered safe because of having specific characterization (Karami et al. 2017). Various important genera of LAB are *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Streptococcus*, *Leuconostoc*, *Pediococcus*, and *Lactococcus* (Fijan 2014). These bacteria help to reduce gastrointestinal disease by means of inducing proper growth of beneficial microorganisms and preventing the population mechanism of pathogens (Caballero et al. 2015). In recent days, probiotics are used not only as a driver of growth but also as a stimulator of the immune system and prevention of many diseases (Fooks and Gibson 2002). Different biochemical tests such as Gram stain; production of indole, catalase, and oxidase; and study of motion, growth at different temperatures (15–37–45), and various fermentations of sugars (sucrose, lactose, maltose, trehalose, galactose, arabinose, mannitol, fructose, and salicin) are studied (Karami et al. 2017).

Various types of epidemiologic and clinical studies require the use of Gram stain that implies smearing a sample onto a slide. Crystal violet is used to stain the smear and kept it for 1 min and then washed with water; after that, application of Gram's iodine is made for 1 min, and then decolorization is done with alcohol. After decolorization, the smear was counterstained with safranine for a minute. Finally, the prepared smear is wiped with water and air-dried. Then the slide is noticed under the microscope (Hossain et al. 2016).

Lactic acid bacteria (LAB) representing the genus of mutuality bacteria persistently colonize the mucosa of the gut that could help to restore the optimum biological balance between the quantity of reactive oxygen species (ROS) and the activity of the antioxidative enzymes synthesized by the bacteria (Pessione 2012). Some species belonging to the genus, for example, *Lactobacillus delbrueckii*, *Lactobacillus gasseri*, and *Lactobacillus acidophilus*, can transform toxic O_2^- to less active H_2O_2:

Table 5.1 List of probiotics with their brand name

Brand name	Component	Manufacturer	References
Activia	*Bifidobacterium lactis* (animalis) DN-173010	Danone, France	Sniffen et al. (2018)
Align	*Bifidobacterium infantis* 35624	Procter and Gamble, USA	
Bioflorin	*Enterococcus faecium* SF 68	Sanofi, Germany	
Bio-K+	*Lactobacillus acidophilus* CL1285, *Lactobacillus casei* Lbc80r, and *Lactobacillus rhamnosus* CLR2	Bio-K+ Intl, Canada	
Culturelle	*Lactobacillus rhamnosus* GG	i-Health, Inc., USA	
Florastor	*Saccharomyces boulardii* CNCM I-745	Biocodex, France	
GynOphilus	*Lactobacillus casei rhamnosus* Lcr35	Biose, France	
Lacteol	*Lactobacillus acidophilus* LB	Mirren, South Africa	
Mutaflor	*Escherichia coli* Nissle 1917	Pharma-Zentiale, Germany	
ProViva	*Lactobacillus plantarum* 299v	Probi AB, Sweden	
ProbioSlim	*Bacillus coagulans* nr	ProbioSlim, Canada, USA	
Actimel	*Lactobacillus casei* Immunitas	Danone, France	Kaur and Das (2011), Mishra et al. (2018)
Aciforce	*Lactococcus lactis*, *Lactobacillus acidophilus*, *Enterococcus faecium*, *Bifidobacterium bifidum*	Biohorma, Netherland	
Bififlor	*Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*	Eko-Bio, Netherland	
Proflora	*Lactobacillus acidophilus*, *Lactobacillus delbrueckii bulgaricus*, *Streptococcus thermophilus*, *Bifidobacterium*	Chefaro, Belgium	
Provie	*Lactobacillus plantarum*	Skanemejerier, Sweden	
Rela	*Lactobacillus reuteri*	Ingman Foods, Finland	
Vitamel	*Lactobacillus casei* GG, *Bifidobacterium bifidum*, *Lactobacillus acidophilus*	Campina, Netherland	
Yosa	*Lactobacillus acidophilus*, *Bifidobacterium lactis*	Bioferme, Finland	

$$2\,O_2^{-} + H^{+} \rightarrow H_2O_2 + O_2$$

These *Lactobacillus* strains liberate H_2O_2 into the extracellular space, which can be detected by suitable qualitative and quantitative estimations.

For the test of indole production, peptone broth is prepared in the amount of 1%. After that, it is sterilized and incubated with the isolated colonies of bacteria, and then incubation is done at 37 °C for 48 h. One ml of Kovacs reagent is added after the incubation process and then gently shaken. Observation of the results is carried out when tubes are allowed to stand. A cherry red ring indicates a positive reaction (MacWilliams 2009).

During the test of oxidase, one drop of 1% Kovacs reagent is added after a loop, and then well-isolated bacterial colony is picked from a fresh (18–24-h culture) plate and stroked on to treated filter paper. Changing of color is noticed to dark purple or blue after 30 s to 1 min, proving the result is positive (Shields and Cathcart 2010).

5.2.3 Role of Probiotics in Host-Gut Microbe Interactions

The gut microbiota plays various essential roles in human health. Beneficial bacteria in the gut has significant role in host health and disease, many of them are obligate anaerobes (Holzapfel et al. 2001). The human gut comprises of an ample amount of diversified microorganisms, bacteria, viruses, archaea, yeast, and fungi, which take over the bowel. Primarily the available probiotics belong to mainly *Saccharomyces* or *Lactobacillus* genera (Fijan 2014). Consequences of subsequent trials showed that these probiotics are advantageous in preventing infectious diarrhea and post-antibacterial diarrhea as colitis due to *Clostridium difficile* (Goldenberg et al. 2017). Research studies till date have developed various strains of probiotics that have the ability to prevent low gastric pH. Considering the gut microbiota ecosystem, the microbiota arranges as a focal ecosystem and changes from one position to another specifically when comparing microbes attached in the intestinal wall or living in mucus, also known as the parietal microbiota, with microbes present in food in transit and stools, known as the luminal microbiota (Zhang et al. 2016).

The composition of the microbiota is vital and individualized based on the pattern of diet, explication to imbibed probiotic bacteria, environmental condition of the intestine, and many other features correlated with the host that will involve "transiently" some other new strains in the ecosystem.

Microbiota in the luminal portion gets modified due to treatment with probiotic, indicating endurance during transportation through the digestive tract. We live in ideal symbiosis of microorganisms, despite continuous exposure of gastrointestinal cells to microbial antigens. In the gastrointestinal tract, the presence of the mucus layer, tight junction proteins, antimicrobial factors, immunoglobulin, and other immunological cells and intraepithelial lymphocytes give rise to complex multidimensional mechanism, thus making the gut barrier function more efficiently (Taverniti and Guglielmetti 2011).

Apart from traditional immune aspects, studies on the interaction between immune response and gut bacteria help to develop more considerably microbial components and receptors that contribute to the control of energy, glucose, and lipid metabolism.

Microbiota present in the gastrointestinal tract influences the typical expansion of gut development because of its capacity to control epithelial cell propagation and apoptosis of host cells (Yan and Polk 2011). Most of the close communication between microbiota and host cells are not known, but a principal machinery includes the formation of short-chain fatty acids resulting from the fermentation of fibers (indigestible polysaccharides) with an anti-inflammatory role (Wells et al. 2016). Thus, microbiota includes short-chain fatty acid production that also maintains intestinal homeostasis in the usual colon and helps in intestinal renovation by the endorsement of cellular differentiation and proliferation. Microbiota also stimulates the development of the nonspecific and specific immune system components. After birth and during our entire life, gut microbiota participate in anti-infectious barrier by successfully inhibiting adherence of pathogen and producing bacteriocins and other toxic metabolites. Spanogiannopoulos et al. (2016) reported that probiotic bacteria in the gut have an influence on the absorption of drug and metabolism in the hepatic region and generate active metabolites that cannot be formed in the liver. Therefore, modulation of the gut microbiota by using probiotics might constitute future direction in developing nutritional, immunological, as well as pharmaceutical contrivances for the maintenance of proper health (Fig. 5.1). In spite of that, more clinical investigations are necessary in turn to filter the clinical suggestion regarding exact probiotic strains to comprehend properly the outcome of substances liberated by probiotic bacteria.

Indigenous microbiota in the gastrointestinal tract is a crucial resource of metabolites, hormones, and neuromodulators which in turn are involved in controlling various important functions of the gut directly and alter the extraintestinal organs such as the liver, cardiovascular system, brain, and kidney indirectly (Park 2018). Considering the physiological point of view, primary connections involving gut microbiota and extraintestinal organs are maintained by the gut-liver axis, therefore closely representing practical and two-way connection between the intestine and liver (Konturek et al. 2018). The liver is continuously exposed to the products of digestion and absorption, and also various gut bacteria, and bacterial products such as lipopolysaccharides (LPS). The gut-liver axis is a potential means to shield the host contrary to detrimental and lethal substances released from the gut, hence retaining the homeostasis of the immune system (Ponziani et al. 2018).

In case the intestinal barrier is damaged and permeability is increased, it might result in dysfunctional intestinal microbiota, and as a result, the liver is continuously exposed to microbial components like pathogen-associated molecular patterns (PAMPS) and damage-associated molecular pattern (DAMP), causing injury. The concept of chronic liver diseases (CLD), in particular liver cirrhosis, has been modified due to the analysis of culture-independent examination of microbiome.

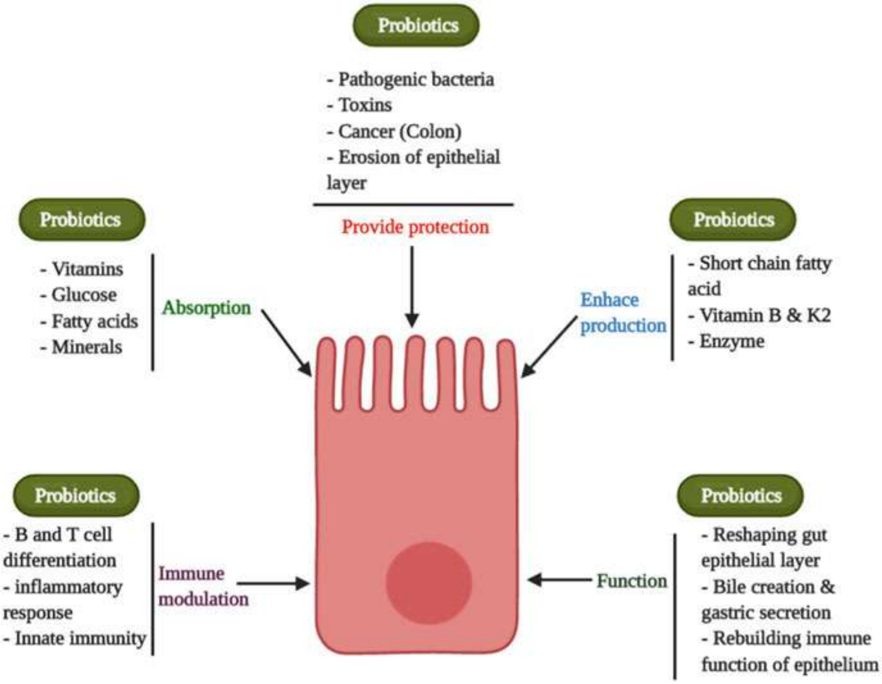


Fig. 5.1 Five principal modes of function of probiotics

Investigational researches show that many diseases related to liver are associated with intestinal dysbiosis (Konturek et al. 2018).

Distinct disruption of gut microbiota influences patients with chronic kidney diseases (CKD). This sequentially impels a series of negative alteration of metabolites, inflammation, uremic toxin (i.e., some components which are generally excreted and filtered by the kidneys) production, and immune suppression and, hence, eventually endorses progressive kidney malfunction and cardiovascular disease (Rossi et al. 2015). After taking a meal, the nutritional ingredients which are failed to be absorbed by the small intestines are in turn fermented by the colonies of bacteria in the large intestine. The two primary types of bacterial fermentation are carbohydrates and protein. Much importance of dietary fiber is assumed in CKD patients on the basis of supplementary benefits with respect to improved reliability of the gastrointestinal wall and might reduce uremic toxins (Birkett et al. 1996).

Various studies suggested the potential function of indigenous gut microbiota in cardiovascular diseases (CVD), atherosclerosis, and heart failure. The innate immune system causes inflammation, and inflammatory markers are associated with obesity and obese-related CVD (Cox et al. 2015). Many investigations reported that intestinal microbiota also influences lipid metabolism and exerts a protective role in atherosclerosis development. Besides, patients with inflammatory bowel disease possessing elevated permeability of their intestinal barrier experience higher risk of chronic heart diseases (CHD), in spite of a lower occurrence of other risk factors (Rogler and Rosano 2014).

Another important area of research is the gut-brain axis, which comprises of bifacial connection between the central and the enteric nervous system, hence creating a connection between the emotional and cognitive centers of the brain with peripheral intestinal function (Carabotti et al. 2015).

Considering a cross talk between the gut and the brain, which is a multifaceted communication system, impacts appropriate preservation of gastrointestinal homeostasis and also possesses various effects on motivations and higher cognitive functions. The central nervous system (CNS), both the brain and spinal cord, the autonomic nervous system (ANS), the enteric nervous system (ENS), and the hypothalamic-pituitary-adrenal axis (HPA) are included in the bidirectional communication network. The influence of intestinal microbiota on the gut-brain axis has been established both clinically and experimentally (Carabotti et al. 2015). Disruption of the gut-brain axis is linked to gut dysbiosis that is also connected with mood disorders (Berrill et al. 2013). Brain-gut dysfunctions are particularly being dominant in irritable bowel syndrome (Koloski et al. 2012). In addition, the impacts of central nervous system (CNS) on the composition or diversity of microbiota are believed to be controlled by a perturbation of the normal luminal/mucosal habitat that can also be reinstated by the utilization of probiotics and probably by nutritional intake.

5.3 Therapeutic Roles of Probiotics in Inflammatory Diseases of the Gut

Several comparative studies revealed differences in α-diversity of gut microbiota along with bacterial stability influencing cellular innate and adaptive immunity (Round and Mazmanian 2009; Kostic et al. 2014). Patients suffering from IBD, more specifically UC (Ott et al. 2004; Nishikawa et al. 2009; Walker et al. 2011) and CD (Ott et al. 2004; Manichanh et al. 2006; Dicksved et al. 2008), have been found with reduced diversity of gut microbes that generate an imbalance between anti- and pro-inflammatory cellular responses, ultimately leading to inflammations in the gut. In turn, the use of probiotic microorganisms can reduce this alarming imbalance situation and finally ameliorate the catastrophic inflammatory diseases (Venturi et al. 1999; Karimi et al. 2005; Carol et al. 2006).

5.3.1 Mechanism of Action at the Cellular and Molecular Level

According to Okamoto and Watanabe (2016), inhibition of BEST2 gene expression may lead to deformity in the mucous formation that best describes the commence of UC, while in CD downregulation of ATG16L1 and XBP1results in dysfunctional Paneth cells (Adolph et al. 2013). In dysbiotic condition, PAMPs have been found to interact with TLRs, NLRs, and Nlrp3 inflammasome to create an active inflammatory circumstance inside the gut epithelium (Zaki et al. 2010; Mukherjee et al. 2018). NOD2 that express as LPS receptor and TLRs follow MyD88-dependent pathway and trigger the activation of NF-κβ, AP-1, and IRF, which are responsible for generating inflammatory stimulation inside the lamina propria (Coleman and Haller 2018). The onset of IBD characterizes as an altered pathophysiological condition of the gut where pathogenic induction may influence dendritic cells (DC) to overproduce Th1, Th2, and Th17 effector T cells (del Carmen 2011). CD differentiation and amplification to Th1 cells mediate enormous production of pro-inflammatory cytokines TNF-α, IFN-γ, and IL-12 (Parronchi et al. 1997), while patients suffering from UC with Th2 cell-type release IL-5, IL-4, and IL-13 (Bamias and Cominelli 2015). Another Th cell population Th17 has been found to significantly contribute in maintaining inflammatory response in IBD with a large amount of IL-17, IL-23, IL-22, and IL-6 cytokines (Sarra et al. 2010; Gálvez 2014). Also, Th1- and Th17-type cytokines such as TNF-α, IL-17, IL-6, IFN-γ, and IL-12 have been found with an additive role in upregulating iNOS expression that finally release huge amount of NO, and in turn mutual effect of NO and pro-inflammatory cytokines leads a continuous noxious state resulting in cellular oxidative stress (Soufli et al. 2015).

Considering these insights of IBD, targeting Th cells might be a fruitful way to combat as Brand and his colleagues in 2009 concluded in their article that targeting Th1 and Th17 is a solution for CD (Brand 2009). Signaling molecules from beneficial bacteria, which are the so-called probiotics, are found to induce and express Treg cells via TLR-, NOD-, and Dectin-1 independent pathways and subsequently reduce the propensity of the effector T cells (Atarashi et al. 2011). Gut commensal bacterium *Bacteroides fragilis* is found to signaled TLR2 to induce Foxp3+ Treg cells and ultimately suppress production of IL-17 that in turn helps in promoting immunogenic tolerance against inflammation (Round and Mazmanian 2010; Coleman and Haller 2018). Similar phenomena are also documented when *Bifidobacterium longum* and *Bifidobacterium infantis* were administered; dramatically, downregulation of pro-inflammatory cytokines IL-17, INF-γ, TNF-α, and IL-8 was found to happen (Soufli et al. 2015). A detailed study of Smits et al. (2005) simplifies the importance of probiotic bacteria in modulating the role of dendritic cell by binding DC-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) and activating Treg cells to produce a rich amount of IL-10, an immunoregulatory and anti-inflammatory cytokine. In support, another study shows bacterium *B. infantis* signals to produce IL-27 that promote IL-10 upregulation and suppress IL-17 (Tanabe et al. 2008). While in search of metabolites of commensal bacteria responsible for such immune-regulative function and developing Treg cells, short-chain fatty acid (SCFA) proves itself as potential inducer (Arpaia et al. 2013). Suppression to activity of Th1 cell is a key role of propionate and butyrate (Vinolo et al. 2011). Moreover, SCFA butyrate also is capable to promote differentiation of Foxp3+ Treg cell (Furusawa et al. 2013; Mukherjee et al. 2018). The most interesting study of Toumi et al. (2014) states probiotics *Bifidobacterium* and *Lactobacillus* also can reduce expression of TLR4 along with iNOS and suggests

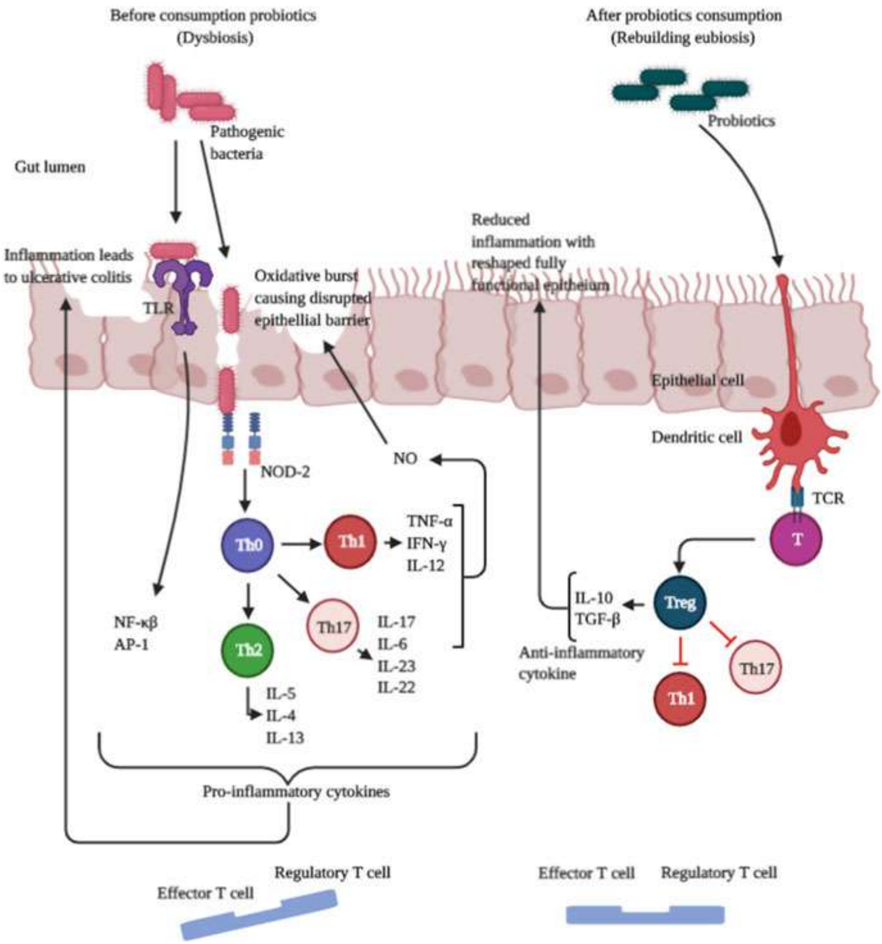


Fig. 5.2 Detail of molecular interaction of pathogenic bacteria and probiotics. The left side is showing pathogenic bacteria in the presence of TLR and NOD-2 receptor that influences differentiation of Th cells into Th1, Th2, and Th17. Cytokines, which are released from effector T cells, are pro-inflammatory in nature. These cytokines affect the gut epithelial layer very badly, leading to ulcerative colitis, bowel disease and even sometimes colorectal cancer. The right side is reflecting the role of probiotics in balancing effector T cells and regulatory T cells by inducing T cells via dendritic cells. Differentiated Treg cells reduce overproduction of effector T cells and in turn increase secretion of anti-inflammatory cytokines, specifically IL-10. Anti-inflammatory cytokines are beneficial as it influences to rebuild the gut epithelial layer and ameliorates inflammatory diseases. *TLR* Toll-like receptor, *NOD-2* nucleotide-binding oligomerization domain-containing protein 2, *IL* interleukin, *Th* helper T cell, *Treg* regulatory T cell, *NF-κβ* nuclear factor κβ, *AP-1* activator protein-1, *TGF-β* transforming growth factor-β, *TCR* T-cell receptor

the potentiality of probiotics as anti-inflammatory agents. Herein, Fig. 5.2 enlightens all the molecular steps driven by probiotics to regulate inflammatory outcomes.

5.4 Therapeutic Roles of Probiotics in Infectious Diseases of the Gut

A healthy gut is a result of commensal gut bacteria and immune cells that have the potential to struggle against pathogens which promote infectious disease. Gut microbes impede the growth of pathogenic organisms by direct inhibition and by regulating nutritive sources (O'Toole and Cooney 2008; Mukherjee et al. 2018), while manipulation of host immunity regulates pathogenesis caused of several infectious invaded pathogens (Clemente et al. 2012). Gut pathogenic bacteria *Salmonella* sp., *Enterococcus* sp. (Kaiser et al. 2013), *Escherichia coli* (Waldman and Balskus 2018), and *Helicobacter pylori* (Sekirov et al. 2010) are found to inhibit and regulate in different studies. A recent study on *Dorea* and *Coprococcus* which belong to the family *Lachnospiraceae* explores its significance to resist *Campylobacter* infection (Bäumler and Sperandio 2016). Thus, dysbiosis in gut microbes for any reason may lead to gastrointestinal infectious diseases. The use of antibiotics is a leading cause that affects the colonization of beneficial bacteria, and in contrary, the growth of resistant pathogenic bacteria is found to increase continuously. In this regard, a study shows how antibiotics alter the microbial colonization and facilitate *Clostridium difficile* to expand vigorously that lead to chronic diarrhea condition (Hickson 2011), while the study of Pavia et al. (1990) concerns how changes in the composition of gut microbiota increase susceptibility to antimicrobial-resistant *Salmonella* strain. Thus, according to Nagpal et al. (2012), application of beneficial bacteria as supplement food habits could be a better solution to keep our gut healthy and protected.

5.4.1 Mechanism of Action at the Cellular and Molecular Level

According to Fig. 5.3, several mechanisms influence gut microbes. Direct antagonism is one of the most common as several studies already emphasized that metabolites released from probiotic bacteria may impede the growth of infectious bacteria. Deoxycholic acid from *Clostridium scindens*, bacteriocins from *Escherichia coli*, and microcins from *Enterococcus faecalis* have a direct inhibitory effect on enteropathogens (Waldman and Balskus 2018), while lactic acid from *Lactobacillus salivarius* shows effectivity on *Helicobacter pylori* (Ryan et al. 2008). Most interestingly, SCFA from *Lachnospiraceae* has a repressive role in the virulent gene of *Salmonella* (Antunes et al. 2014). In similar example, mutagenic autoinducer (AI)-2 from *Ruminococcus obeum* is also found to inhibit the expression of the virulent gene of *Vibrio cholerae* (Waldman and Balskus 2018).

Apart from these human cases, several other bacteriocins like UCC118 from *Lactobacillus salivarius* have an oppressive role in *Salmonella typhimurium*

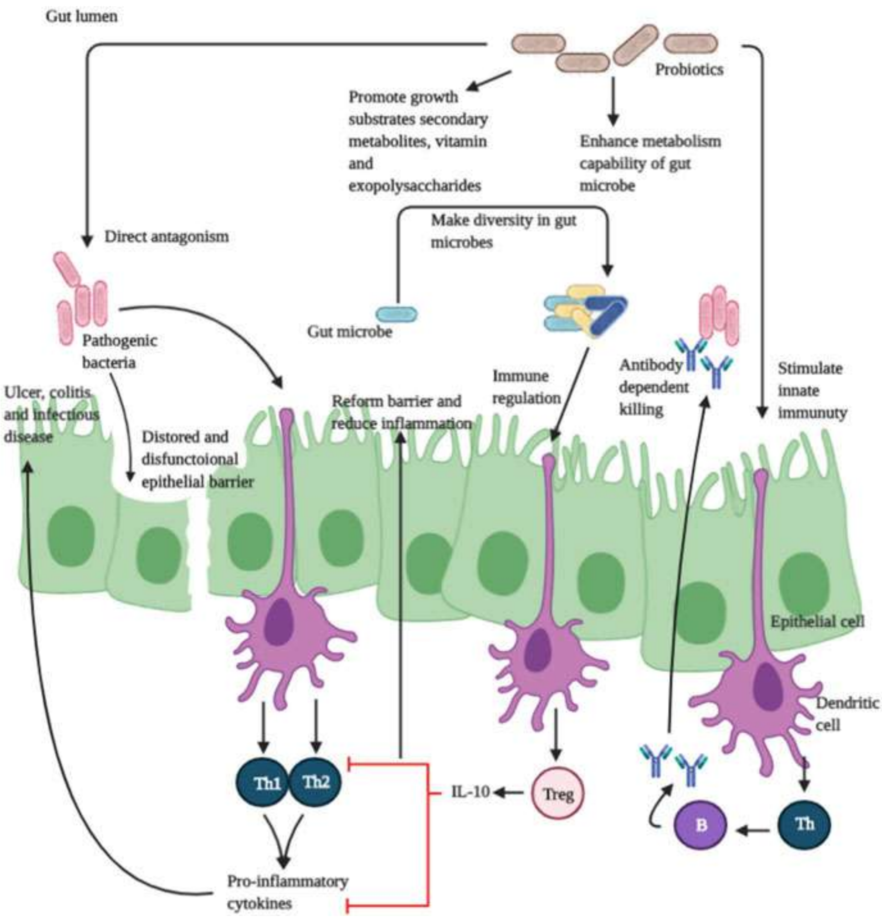


Fig. 5.3 Molecular mechanism of probiotics ameliorating infectious diseases. Probiotics have several roles as it can directly inhibit pathogenic bacteria and can stimulate host innate immunity to reduce pathogen levels. Probiotics also can promote the growth of gut microbes by enriching the gut with metabolites to reduce competition. Even probiotics alter gene expression patterns of gut microbes to make diversity. Probiotics also modulate host immunity and balance pro-inflammatory and anti-inflammatory cytokines, resulting in ameliorated infectious and inflammatory diseases. *Th* helper T cell, *IL* interleukin, *B* B cell

infection on mouse model (O'Toole and Cooney 2008). According to O'Toole and Cooney (2008), a proper combination of probiotics can competitively exclude pathogenic bacteria as Collado et al. (2008) found in his study the elimination strategy of *Enterobacter sakazakii*.

Coexistence of probiotic bacteria *Bifidobacterium longum* and *Lactobacillus casei* with normal gut microbiota *Bacteroides thetaiotaomicron* affects their uniformity in carbohydrate metabolism that leads to modification in gene expression pattern of *Bacteroides thetaiotaomicron* (Sonnenburg et al. 2006). Thus, Sonnenburg and his colleague on their study conclude that the utilization of probiotics can reduce competition between gut microbes on nutrition availability by expanding the range of diverse carbohydrate metabolism capabilities (Sonnenburg et al. 2006). Besides these, metabolic profiling is another strategy where probiotics promote better survival of gut microbes with reduced competition as LAB is found to produce exopolysaccharides and a variety of secondary metabolites like N-acetyl metabolites, unconjugated bile acids, choline, acetate, ethanol, and tauro-conjugated bile acids that are highly essential for healthier growth of gut microbes (O'Toole and Cooney 2008). Moreover, *Lactococcus lactis*, *Lactobacillus gasseri*, and *Bifidobacterium adolescentis* are also crucial to supply adequate vitamins, specifically riboflavin and folate (Gu and Li 2016).

Immune stimulation or regulating host immunity may alter the inflammatory atmosphere to protect the host and support survival and fitness. Polysaccharide A (PSA) from *Bacteroides fragilis* sensitizes dendritic cells and differentiates T lymphocytes from Treg cells that in turn induce IL-10 secretion and downregulate pro-inflammatory cytokines TNF-α, IL-17, and IL-23 (Round and Mazmanian 2010). IgA also is found to reduce pro-inflammatory expression that helps to maintain a balanced relationship between host and microbes (Peterson et al. 2007). Most strikingly, the induction of butyrate activates M2 macrophages via H3K9 acetylation and reduces inflammation that finally helps to repair the damaged host tissues (Spiljar et al. 2017), whereas angiogenin (Ang1, Ang4) from Paneth cells after the influence of *Bacteroides thetaiotaomicron* may regulate host innate immunity and express bactericidal effect. A study of Hooper et al. (2003) exhibit the antibacterial role of Ang4 on *Enterococcus faecalis* and *Listeria monocytogenes* in mouse model.

5.5 Therapeutic Promises of Probiotics

Studies already discussed earlier clearly depict probiotics have promising role to reduce pathogenic bacterial influence on the gut as shown in Table 5.2 and establish a balanced relationship among pro-inflammatory and anti-inflammatory cytokines subject to maintain a physiologically and functionally healthy gut. Antibiotics are long being used to treat pathogenic bacterial infections; it has a profound effect on filariasis caused by helminth parasites. Metronidazole is one of the common therapeutics used against filariasis that also has a pivotal role in killing anaerobic gut microbes, especially *Bacteroides fragilis*. But prolonged use of metronidazole causes several neurotoxic effects that hamper gut physiology. On the other hand, clindamycin targets both anaerobes and Gram-positive bacteria along with having a high absorption rate through the intestine which may lead to adverse effect on the gut. Treatment with probiotics firstly influence gut microbes that increase diversity and reduce competition to reach the maximum growth of gut microbes. Most interestingly, antibody-associated disease (AAD) mainly in children is also found to ameliorate after the use of probiotics *Lactobacillus* sp. and *Saccharomyces boulardii* (McFarland 2006). Recent studies found out antibiotics have a direct

Table 5.2 List of probiotics with their therapeutic details for selective diseases

Type of disease		Probiotics used	Dosage	Duration of treatment	References
Antibiotic-associated diarrhea (AAD)	Clarithromycin, amoxicillin, and omeprazole induced diarrhea	*Saccharomyces boulardii*	Two times per day (500 mg)	14 days	Duman et al. (2005)
	–	*Lactobacillus acidophilus*, *Bifidobacterium animalis lactis*, and *Streptococcus thermophilus*	One time per day (10^9 CFU)	12 days	Conway et al. (2007)
	Amoxicillin induced diarrhea	*Lactobacillus acidophilus* and *Lactobacillus bulgaricus*	Four times per day (5.1×10^8 CFU)	10 days	Tankanow et al. (1990)
	Levofloxacin, and amoxicillin induced side effect	*Lactobacillus reuteri*	Three times per day (1×10^8 CFU)	14 days	Ojetti et al. (2012)
	Clarithromycin, amoxicillin, and omeprazole induced adverse effect	*Saccharomyces boulardii*	Two times per day (250 mg)	14 days	Zojaji et al. (2013)
Clostridium difficile infections (CDI)		*Saccharomyces boulardii*, *Lactobacillus acidophilus*, and *Lactobacillus casei*	10–50 billion CFU per day	–	Goldenberg et al. (2017)
		Saccharomyces boulardii, *Lactobacillus* sp., *Bifidobacterium* sp., and *Streptococcus* sp.	–	–	Mills et al. (2018)
Inflammatory bowel disease (IBD)	Crohn's disease	*Lactobacillus rhamnosus* GG	–	6 months	Gupta et al. (2000)
		Saccharomyces boulardii	–	6 months	Guslandi et al. (2000)
	Ulcerative colitis	*Escherichia coli*	–	12 weeks	Kruis et al. (1997)
		Lactobacillus plantarum	–	–	Kordecki and Niedzielin (2001)

(continued)

Table 5.2 (continued)

Type of disease	Probiotics used	Dosage	Duration of treatment	References
Helicobacter pylori eradication	*Lactobacillus reuteri*	10^8 CFU per day	7 days	Scaccianoce et al. (2008)
	Lactobacillus gasseri OLL2716	5×10^8 CFU per gram cheese	1 year	Boonyaritichaikij et al. (2009)
	Lactobacillus casei, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, and *Bifidobacterium breve*	1×10^8 CFU per day	14 days	Shavakhi et al. (2013)
	Lactobacillus rhamnosus GG	10^8–10^{10} CFU	14 days	Hauser et al. (2015)
Irritable bowel syndrome (IBS)	*Lactobacillus rhamnosus* GG, *Lactobacillus rhamnosus LC705*, *Bifidobacterium breve Bb99*, and *Propionibacterium freudenreichii*	–	6 months	Kajander et al. (2005)
	Bifidobacterium infantis	–	4 weeks	Whorwell et al. (2006)
	Bifidobacterium animalis DN-173 010	–	6 weeks	Guyonnet et al. (2007)
Necrotizing enterocolitis (NEC)	*Lactobacillus rhamnosus GG*	10^9 CFU per day	34 weeks	Al-Hosni et al. (2012)

impact on developing *Clostridium difficile* infection (CDI) (Nitzan et al. 2013). The fecal microbiota transplantation (FMT) technique is well known to be successful against CDI along with IBD as a study of Choi and Cho (2016) found enhancing *Firmicutes* richness results in reduced inflammation.

Moreover, antibiotics like vancomycin have been reported to develop allergic airway inflammation (AAI), while probiotics promote necessary vitamins, metabolites, and carbohydrates for better growth of gut microbes that in turn reduce inflammation. Apart from reducing the diversity of gut microbes, prolonged and inappropriate use of antibiotics is also responsible for generating resistant pathogenic bacteria that leads to the threatened consequence of host life. Phage-based therapy is now in trend to treat multidrug-resistant strain (Mukherjee et al. 2018). Primarily stools are examined for microbes and then target specific phages are applied.

Nonsteroidal anti-inflammatory drugs (NSAID) are widespread for their tremendous analgesic role. Besides it, they have potentiality to prevent blood clotting and minimize inflammation. More specifically, they regulate the activity of isoenzymes of cyclooxygenase (COX-1 and COX-2). Even so, sometimes NSAID causes serious damage in the gut, specifically in the intestine. To date, there are no available prophylactic agents that can combat such damages, but a study of Otani et al. (2017) found out probiotics *Lactobacillus* and *Bifidobacterium* have the potential to reduce ulcers caused by NSAID.

Evidences explore probiotics also have anti-colorectal cancer properties that limit specific bacterial population which have an influence on the rising levels of carcinogen (Markowiak and Śliżewska 2017) and that different probiotics from other available therapeutics have both anti-inflammatory and anti-colon cancer properties. Considering all the discussions above, it can be concluded that probiotics are the only hope as a prophylactic agent that has the ability to keep our gut well and good.

5.6 Prospects and Challenges

Although naturally obtained probiotics have sufficient potency, the use of prebiotics greatly boosts up the power of probiotics. Prebiotics are indigestible fibers that upon processed by bacteria produce short-chain fatty acids which in benefit facilitate the growth of gut microbes along with the help to retain the gut healthy (Davani-Davari et al. 2019). Thus, synbiotic is now in high demand as it provides living beneficial microbes along with supplementary nutrients that are ready to ferment (Pandey et al. 2015). Multistrain probiotic is an upgrade in probiotic therapy that also provides enhanced activity as it includes synergistic interaction of more than one bacterium. For example, the combined effect of *Lactobacillus salivarius* and *Bifidobacterium breve* shows proficiency in activity to decrease pro-inflammatory cytokines and increase anti-inflammatory cytokines than individual effect (Drago et al. 2015). Such advanced approaches are very useful, quickly upregulate mucus production, reform the gut epithelium, and restore barrier function (Hwang et al. 2017).

Combined effects of probiotic and antibiotic are also found to be very effective when treated against bacteria that cause burn disease. Tetracycline in conjugate with probiotic *Lactobacillus plantarum* 299v shows high efficiency in diminishing multidrug resistance *Pseudomonas aeruginosa* (Moghadam et al. 2018). Apart from these natural probiotics, genetically engineered one is now in most an interesting aspect as it modifies the bacteria for specific target. Genetically engineered *Lactobacillus lactis* IL1403 specifically express antimicrobial peptide alyteserin and A3APO to counter *Escherichia coli* and *Salmonella* sp. (Mazhar et al. 2020). Advance application of genetically modified bacteria is now skyrocketing, replacing the vaccine therapy as vaccines have chances of virulence reversal where modified bacteria are only used as a vector to carry immunogenic substances so no chances of side effects (Tarahomjoo 2012). Most strikingly, some studies achieve success in probiotics after regulating the virulent gene expression of pathogenic bacteria (Galván-Moroyoqui et al. 2008).

To overcome challenges like short shelf life, lower survival rate from several environmental factors, many strategies and modifications have been programmed. As reported, first-generation probiotics have very low shelf life, that is, only 2 weeks for *B. longum* (Takahashi et al. 2004). The addition of sodium alginate shows better results in enhancing shelf life to 6 months (Klayraung et al. 2009). At present, fourth-generation probiotics have been modified with higher survival as it encapsulated with biofilm of bacterial cells (Salas-Jara et al. 2016).

5.7 Conclusion and Future Directions

In the twenty-first century, inflammatory and infectious diseases related to the gut are an ever-growing health issue. Countries that lead advanced lifestyle are more susceptible to such diseases. Even modern food habits are also a very vital aspect that downgrade gut health. Though there are several drugs available in the market like antibiotics and NSAIDs potent for such diseases, in turn, they cause several side effects too. In this scenario, probiotics give hope as opportunistic therapeutic agents with no side effects. Moreover, several advanced strategies are capable to enhance the effectiveness of probiotics. Even the modern genetically modified probiotics are highly specific for the target. Despite several achievement stories, there still have several gaps in understanding the mechanics inside the success. This is the time to crack the basic biology works to ameliorate infectious disease and enrich the knowledge on components that modulate the expression pattern of selective genes of gut microbes for better survival. These findings will help us to understand the complexity of future diseases and guide us to modify specific prophylactic agents to combat such situations.

Modern approaches still need several modifications as some studies figure out multistrain failed to prove their synergistic effect (Trush et al. 2020). Even combined therapy of antibiotics with probiotics also faced several failure reports (Moghadam et al. 2018). Thus, proper guidance and detailed study on probiotics and their target are still required to overcome such failure. Success on such drawbacks will motivate researchers to formulate more strategies that empower probiotics more to keep a smile forever on our gut.

Acknowledgments The authors gratefully acknowledge BioRender.com used for drawing the figures. All the related uncited articles which have not been incorporated due to space limitation are duly acknowledged. RP thanks the Department of Higher Education, Government of West Bengal, for his merit-cum-means fellowship.

References

Adolph TE, Tomczak MF, Niederreiter L et al (2013) Paneth cells as a site of origin for intestinal inflammation. Nature 503:272–276. https://doi.org/10.1038/nature12599

Al-Hosni M, Duenas M, Hawk M et al (2012) Probiotics-supplemented feeding in extremely low-birth-weight infants. J Perinatol 32:253–259. https://doi.org/10.1038/jp.2011.51

Antunes LCM, McDonald JAK, Schroeter K et al (2014) Antivirulence activity of the human gut metabolome. MBio 5:e01183. https://doi.org/10.1128/mBio.01183-14

Arpaia N, Campbell C, Fan X et al (2013) Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature 504:451–455. https://doi.org/10.1038/nature12726

Atarashi K, Tanoue T, Shima T et al (2011) Induction of colonic regulatory T cells by indigenous Clostridium species. Science 331:337–341. https://doi.org/10.1126/science.1198469

Backhed F, Ley RE, Sonnenburg JL (2005) DA Peterson & JI Gordon. Science 307:1915. https://doi.org/10.1126/science.1104816

Bamias G, Cominelli F (2015) Role of Th2 immunity in intestinal inflammation. Curr Opin Gastroenterol 31:471. https://doi.org/10.1097/MOG.0000000000000212

Barra M, Danino T, Garrido D (2020) Engineered probiotics for detection and treatment of inflammatory intestinal diseases. Front Bioeng Biotechnol 8:265. https://doi.org/10.3389/fbioe.2020.00265

Bäumler AJ, Sperandio V (2016) Interactions between the microbiota and pathogenic bacteria in the gut. Nature 535:85–93. https://doi.org/10.1038/nature18849

Belizário JE, Faintuch J (2018) Microbiome and gut dysbiosis. In: Metabolic interaction in infection. Springer, New York, pp 459–476. https://doi.org/10.1007/978-3-319-74932-7_13

Berrill JW, Gallacher J, Hood K et al (2013) An observational study of cognitive function in patients with irritable bowel syndrome and inflammatory bowel disease. Neurogastroenterol Motil 25:918. https://doi.org/10.1111/nmo.12219

Birkett A, Muir J, Phillips J et al (1996) Resistant starch lowers fecal concentrations of ammonia and phenols in humans. Am J Clin Nutr 63:766–772. https://doi.org/10.1093/ajcn/63.5.766

Boonyaritichaikij S, Kuwabara K, Nagano J et al (2009) Long-term administration of probiotics to asymptomatic pre-school children for either the eradication or the prevention of Helicobacter pylori infection. Helicobacter 14:202–207. https://doi.org/10.1111/j.1523-5378.2009.00675.x

Brand S (2009) Crohn's disease: Th1, Th17 or both? The change of a paradigm: new immunological and genetic insights implicate Th17 cells in the pathogenesis of Crohn's disease. Gut 58: 1152–1167. https://doi.org/10.1136/gut.2008.163667

Caballero S, Carter R, Ke X et al (2015) Distinct but spatially overlapping intestinal niches for vancomycin-resistant Enterococcus faecium and carbapenem-resistant Klebsiella pneumoniae. PLoS Pathog 11:e1005132. https://doi.org/10.1371/journal.ppat.1005132

Cani PD (2018) Human gut microbiome: hopes, threats and promises. Gut 67:1716–1725. https://doi.org/10.1136/gutjnl-2018-316723

Carabotti M, Scirocco A, Maselli MA, Severi C (2015) The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. Ann Gastroenterol 28:203–209

Carol M, Borruel N, Antolin M et al (2006) Modulation of apoptosis in intestinal lymphocytes by a probiotic bacteria in Crohn's disease. J Leukoc Biol 79:917–922. https://doi.org/10.1189/jlb.0405188

Choi HH, Cho Y-S (2016) Fecal microbiota transplantation: current applications, effectiveness, and future perspectives. Clin Endosc 49:257. https://doi.org/10.5946/ce.2015.117

Clemente JC, Ursell LK, Parfrey LW, Knight R (2012) The impact of the gut microbiota on human health: an integrative view. Cell 148:1258–1270. https://doi.org/10.1016/j.cell.2012.01.035

Coleman OI, Haller D (2018) Bacterial signaling at the intestinal epithelial interface in inflammation and cancer. Front Immunol 8:1927. https://doi.org/10.3389/fimmu.2017.01927

Collado MC, Isolauri E, Salminen S (2008) Specific probiotic strains and their combinations counteract adhesion of Enterobacter sakazakii to intestinal mucus. FEMS Microbiol Lett 285: 58–64. https://doi.org/10.1111/j.1574-6968.2008.01211.x

Conway S, Hart A, Clark A, Harvey I (2007) Does eating yogurt prevent antibiotic-associated diarrhoea? A placebo-controlled randomised controlled trial in general practice. Br J Gen Pract 57:953–959. https://doi.org/10.3399/096016407782604811

Cox AJ, West NP, Cripps AW (2015) Obesity, inflammation, and the gut microbiota. Lancet Diabetes Endocrinol 3:207–215. https://doi.org/10.1016/S2213-8587(14)70134-2

Davani-Davari D, Negahdaripour M, Karimzadeh I et al (2019) Prebiotics: definition, types, sources, mechanisms, and clinical applications. Foods 8:92. https://doi.org/10.3390/foods8030092

del Carmen S (2011) de Moreno A, Miyoshi A, Santos Rocha C, Azevedo V, Le Blanc JG. Potential application probiotics Prev Treat Inflamm 2011:1–13. DOI: https://doi.org/10.1155/2011/841651

Den Besten G, van Eunen K, Groen AK et al (2013) The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res 54:2325–2340. https://doi.org/10.1194/jlr.R036012

Dethlefsen L, Relman DA (2011) Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. Proc Natl Acad Sci 108:4554–4561. https://doi.org/10.1073/pnas.1000087107

Dicksved J, Halfvarson J, Rosenquist M et al (2008) Molecular analysis of the gut microbiota of identical twins with Crohn's disease. ISME J 2:716–727. https://doi.org/10.1038/ismej.2008.37

Drago L, De Vecchi E, Gabrieli A et al (2015) Immunomodulatory effects of Lactobacillus salivarius LS01 and Bifidobacterium breve BR03, alone and in combination, on peripheral blood mononuclear cells of allergic asthmatics. Allergy, Asthma Immunol Res 7:409–413. https://doi.org/10.4168/aair.2015.7.4.409

Duman DG, Bor S, Özütemiz Ö et al (2005) Efficacy and safety of Saccharomyces boulardii in prevention of antibiotic-associated diarrhoea due to Helicobacterpylori eradication. Eur J Gastroenterol Hepatol 17:1357–1361. https://doi.org/10.1097/00042737-200512000-00015

FAO/WHO (2001) Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria. Rep. from FAO/WHO Expert Consult, pp 1–4

Fernández A, del Campo P, De Orta PA, Straface JI et al (2019) The use of probiotic therapy to modulate the gut microbiota and dendritic cell responses in inflammatory bowel diseases. Med Sci 7:33. https://doi.org/10.3390/medsci7020033

Fijan S (2014) Microorganisms with claimed probiotic properties: an overview of recent literature. Int J Environ Res Public Health 11:4745–4767. https://doi.org/10.3390/ijerph110504745

Fooks LJ, Gibson GR (2002) Probiotics as modulators of the gut flora. Br J Nutr 88:39–49. https://doi.org/10.1079/BJN2002628

Furusawa Y, Obata Y, Fukuda S et al (2013) Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature 504:446–450. https://doi.org/10.1038/nature12721

Galván-Moroyoqui JM, Carmen Dominguez-Robles M, Franco E, Meza I (2008) The interplay between Entamoeba and enteropathogenic bacteria modulates epithelial cell damage. PLoS Negl Trop Dis 2:e266. https://doi.org/10.1371/journal.pntd.0000266

Gálvez J (2014) Role of Th17 cells in the pathogenesis of human IBD. Int Sch Res Not 2014: 928461. https://doi.org/10.1155/2014/928461
Gensollen T, Iyer SS, Kasper DL, Blumberg RS (2016) How colonization by microbiota in early life shapes the immune system. Science 352:539–544. https://doi.org/10.1126/science.aad9378
Goldenberg JZ, Yap C, Lytvyn L et al (2017) Probiotics for the prevention of Clostridium difficile-associated diarrhea in adults and children. Cochrane Database Syst Rev 12:CD006095. https://doi.org/10.1002/14651858.CD006095.pub4
Gu Q, Li P (2016) Biosynthesis of vitamins by probiotic bacteria. Probiotics prebiotics. Hum Nutr Health. https://doi.org/10.5772/63117
Gupta P, Andrew H, Kirschner BS, Guandalini S (2000) Is Lactobacillus GG helpful in children with Crohn's disease? Results of a preliminary, open-label study. J Pediatr Gastroenterol Nutr 31:453–457. https://doi.org/10.1097/00005176-200010000-00024
Guslandi M, Mezzi G, Sorghi M, Testoni PA (2000) Saccharomyces boulardii in maintenance treatment of Crohn's disease. Dig Dis Sci 45:1462–1464. https://doi.org/10.1023/a:1005588911207
Guyonnet D, Chassany O, Ducrotte P et al (2007) Effect of a fermented milk containing Bifidobacterium animalis DN-173 010 on the health-related quality of life and symptoms in irritable bowel syndrome in adults in primary care: a multicentre, randomized, double-blind, controlled trial. Aliment Pharmacol Ther 26:475–486. https://doi.org/10.1111/j.1365-2036.2007.03362.x
Hauser G, Salkic N, Vukelic K et al (2015) Probiotics for standard triple Helicobacter pylori eradication: a randomized, double-blind, placebo-controlled trial. Medicine 94:e685. https://doi.org/10.1097/MD.0000000000000685
Hickson M (2011) Probiotics in the prevention of antibiotic-associated diarrhoea and Clostridium difficile infection. Ther Adv Gastroenterol 4:185–197. https://doi.org/10.1177/1756283X11399115
Holzapfel WH, Haberer P, Geisen R et al (2001) Taxonomy and important features of probiotic microorganisms in food and nutrition. Am J Clin Nutr 73:365–373. https://doi.org/10.1093/ajcn/73.2.365s
Hooper LV, Stappenbeck TS, Hong CV, Gordon JI (2003) Angiogenins: a new class of microbicidal proteins involved in innate immunity. Nat Immunol 4:269–273. https://doi.org/10.1038/ni888
Hossain MS, Al-Bari MAA, Wahed MII (2016) Biochemical characterization of probiotics available in Bangladesh. J Sci Res 8:101–108. https://doi.org/10.3329/jsr.v8i1.25299
Hwang IY, Koh E, Wong A et al (2017) Engineered probiotic Escherichia coli can eliminate and prevent Pseudomonas aeruginosa gut infection in animal models. Nat Commun 8:1–11. https://doi.org/10.1038/ncomms15028
Jakobsson HE, Jernberg C, Andersson AF et al (2010) Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. PLoS One 5:e9836. https://doi.org/10.1371/journal.pone.0009836
Jernberg C, Löfmark S, Edlund C, Jansson JK (2007) Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. ISME J 1:56–66. https://doi.org/10.1038/ismej.2007.3
Kaiser BLD, Li J, Sanford JA et al (2013) A multi-omic view of host-pathogen-commensal interplay in Salmonella-mediated intestinal infection. PLoS One 8:e67155. https://doi.org/10.1371/journal.pone.0067155
Kajander K, Hatakka K, Poussa T et al (2005) A probiotic mixture alleviates symptoms in irritable bowel syndrome patients: a controlled 6-month intervention. Aliment Pharmacol Ther 22:387–394. https://doi.org/10.1111/j.1365-2036.2005.02579.x
Karami S, Roayaei M, Hamzavi H et al (2017) Isolation and identification of probiotic Lactobacillus from local dairy and evaluating their antagonistic effect on pathogens. Int J Pharm Investig 7:137. https://doi.org/10.4103/jphi.JPHI_8_17

Karimi O, Peña AS, Bodegraven AA (2005) Probiotics (VSL# 3) in arthralgia in patients with ulcerative colitis and Crohn's disease: a pilot study. Drugs Today 41:453–460. https://doi.org/10.1358/dot.2005.41.7.917341

Kaur S, Das M (2011) Functional foods: an overview. Food Sci Biotechnol 20:861. https://doi.org/10.1007/s10068011-0121-7

Klayraung S, Viernstein H, Okonogi S (2009) Development of tablets containing probiotics: effects of formulation and processing parameters on bacterial viability. Int J Pharm 370:54–60. https://doi.org/10.1016/j.ijpharm.2008.11.004

Koloski NA, Jones M, Kalantar J et al (2012) The brain–gut pathway in functional gastrointestinal disorders is bidirectional: a 12-year prospective population-based study. Gut 61:1284–1290. https://doi.org/10.1136/gutjnl-2011-300474

Konturek PC, Harsch IA, Konturek K et al (2018) Gut–liver axis: how do gut bacteria influence the liver? Med Sci 6:79. https://doi.org/10.3390/medsci6030079/

Kordecki HJ, Niedzielin K (2001) May the enrichment of colon microflora with Lactobacillus plantarum improve the results of treatment of irritable bowel syndrome and/or ulcerative colitis. Gut 49:2880. https://doi.org/10.3390/medsci6030079

Kostic AD, Xavier RJ, Gevers D (2014) The microbiome in inflammatory bowel disease: current status and the future ahead. Gastroenterology 146:1489–1499. https://doi.org/10.1053/j.gastro.2014.02.009

Kruis W, Schütz E, Fric P et al (1997) Double-blind comparison of an oral Escherichia coli preparation and mesalazine in maintaining remission of ulcerative colitis. Aliment Pharmacol Ther 11:853–858. https://doi.org/10.1046/j.1365-2036.1997.00225.x

MacWilliams MP (2009) Indole test protocol. American Society for Microbiology, Washington, DC

Manichanh C, Rigottier-Gois L, Bonnaud E et al (2006) Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. Gut 55:205–211. https://doi.org/10.1136/gut.2005.073817

Markowiak P, Śliżewska K (2017) Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients 9:1021. https://doi.org/10.3390/nu9091021

Mazhar SF, Afzal M, Almatroudi A et al (2020) The prospects for the therapeutic implications of genetically engineered probiotics. J Food Qual 2020:9676452. https://doi.org/10.1155/2020/9676452

McFarland LV (2006) Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of Clostridium difficile disease. Am J Gastroenterol 101:812–822. https://doi.org/10.1111/j.1572-0241.2006.00465.x

Mills JP, Rao K, Young VB (2018) Probiotics for prevention of Clostridium difficile infection. Curr Opin Gastroenterol 34:3. https://doi.org/10.1097/MOG.0000000000000410

Mishra SS, Behera PK, Kar B, Ray RC (2018) Advances in probiotics, prebiotics and nutraceuticals. In: Innovations in technologies for fermented food and beverage industries. Springer, New York, pp 121–141. https://doi.org/10.1007/978-3-319-74820-7_7

Moghadam SS, Khodaii Z, Zadeh SF et al (2018) Synergistic or antagonistic effects of probiotics and antibiotics-alone or in combination-on antimicrobial-resistant Pseudomonas aeruginosa isolated from burn wounds. Arch Clin Infect Dis 13:e63121. https://doi.org/10.5812/archcid.63121

Mukherjee S, Joardar N, Sengupta S, Babu SPS (2018) Gut microbes as future therapeutics in treating inflammatory and infectious diseases: lessons from recent findings. J Nutr Biochem 61:111–128. https://doi.org/10.1016/j.jnutbio.2018.07.010

Nagpal R, Kumar A, Kumar M et al (2012) Probiotics, their health benefits and applications for developing healthier foods: a review. FEMS Microbiol Lett 334:1–15. https://doi.org/10.1111/j.1574-6968.2012.02593.x

Natividad JMM, Verdu EF (2013) Modulation of intestinal barrier by intestinal microbiota: pathological and therapeutic implications. Pharmacol Res 69:42–51. https://doi.org/10.1016/j.phrs.2012.10.007

Nishikawa J, Kudo T, Sakata S et al (2009) Diversity of mucosa-associated microbiota in active and inactive ulcerative colitis. Scand J Gastroenterol 44:180–186. https://doi.org/10.1080/00365520802433231

Nitzan O, Elias M, Chazan B et al (2013) Clostridium difficile and inflammatory bowel disease: role in pathogenesis and implications in treatment. World J Gastroenterol 19:7577. https://doi.org/10.3748/wjg.v19.i43.7577

O'Toole PW, Cooney JC (2008) Probiotic bacteria influence the composition and function of the intestinal microbiota. Interdiscip Perspect Infect Dis 2008:175285. https://doi.org/10.1155/2008/175285

Ojetti V, Bruno G, Ainora ME et al (2012) Impact of Lactobacillus reuteri supplementation on anti-Helicobacter pylori levofloxacin-based second-line therapy. Gastroenterol Res Pract 2012: 740381. https://doi.org/10.1155/2012/740381

Okamoto R, Watanabe M (2016) Role of epithelial cells in the pathogenesis and treatment of inflammatory bowel disease. J Gastroenterol 51:11–21. https://doi.org/10.1007/s00535-015-1098-4

Otani K, Tanigawa T, Watanabe T et al (2017) Microbiota plays a key role in non-steroidal anti-inflammatory drug-induced small intestinal damage. Digestion 95:22–28. https://doi.org/10.1159/000452356

Ott SJ, Musfeldt M, Wenderoth DF et al (2004) Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. Gut 53:685–693. https://doi.org/10.1136/gut.2003.025403

Pandey KR, Naik SR, Vakil BV (2015) Probiotics, prebiotics and synbiotics-a review. J Food Sci Technol 52:7577–7587. https://doi.org/10.1007/s13197-015-1921-1

Park W (2018) Gut microbiomes and their metabolites shape human and animal health. J Microbiol 56:151–153. https://doi.org/10.1007/s12275-018-0577-8

Parronchi P, Romagnani P, Annunziato F et al (1997) Type 1 T-helper cell predominance and interleukin-12 expression in the gut of patients with Crohn's disease. Am J Pathol 150:823–832

Pavia AT, Shipman LD, Wells JG et al (1990) Epidemiologic evidence that prior antimicrobial exposure decreases resistance to infection by antimicrobial-sensitive Salmonella. J Infect Dis 161:255–260. https://doi.org/10.1093/infdis/161.2.255

Pessione E (2012) Lactic acid bacteria contribution to gut microbiota complexity: lights and shadows. Front Cell Infect Microbiol 2:86. https://doi.org/10.3389/fcimb.2012.00086

Peterson DA, McNulty NP, Guruge JL, Gordon JI (2007) IgA response to symbiotic bacteria as a mediator of gut homeostasis. Cell Host Microbe 2:328–339. https://doi.org/10.1016/j.chom.2007.09.013

Ponziani FR, Zocco MA, Cerrito L et al (2018) Bacterial translocation in patients with liver cirrhosis: physiology, clinical consequences, and practical implications. Expert Rev Gastroenterol Hepatol 12:641–656. https://doi.org/10.1080/17474124.2018.1481747

Rogler G, Rosano G (2014) The heart and the gut. Eur Heart J 35:426–430. https://doi.org/10.1093/eurheartj/eht271

Rossi M, Johnson DW, Campbell KL (2015) The kidney–gut axis: implications for nutrition care. J Ren Nutr 25:399–403. https://doi.org/10.1053/j.jrn.2015.01.017

Round JL, Mazmanian SK (2009) The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 9:313–323. https://doi.org/10.1038/nri2515

Round JL, Mazmanian SK (2010) Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc Natl Acad Sci 107:12204–12209. https://doi.org/10.1073/pnas.0909122107

Ryan KA, Daly P, Li Y et al (2008) Strain-specific inhibition of Helicobacter pylori by Lactobacillus salivarius and other lactobacilli. J Antimicrob Chemother 61:831–834. https://doi.org/10.1099/jmm.0.009407-0

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

Sarra M, Pallone F, MacDonald TT, Monteleone G (2010) Il-23/Il-17 axis in IBD. Inflamm Bowel Dis 16:1808–1813. https://doi.org/10.1002/ibd.21248

Scaccianoce G, Zullo A, Hassan C et al (2008) Triple therapies plus different probiotics for. Eur Rev Med Pharmacol Sci 12:251–256

Sekirov I, Russell SL, Antunes LCM, Finlay BB (2010) Gut microbiota in health and disease. Physiol Rev 90:859–904. https://doi.org/10.1152/physrev.00045.2009

Shavakhi A, Tabesh E, Yaghoutkar A et al (2013) The effects of multistrain probiotic compound on bismuth-containing quadruple therapy for helicobacter pylori infection: a randomized placebo-controlled triple-blind study. Helicobacter 18:280–284. https://doi.org/10.1111/hel.12047

Shields P, Cathcart L (2010) Oxidase test protocol. ASM, Washington, DC

Smits HH, Engering A, van der Kleij D 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. J Allergy Clin Immunol 115:1260–1267. https://doi.org/10.1016/j.jaci.2005.03.036

Sniffen JC, McFarland LV, Evans CT, Goldstein EJC (2018) Choosing an appropriate probiotic product for your patient: An evidence-based practical guide. PLoS One 13:e0209205. https://doi.org/10.1371/journal.pone.0209205

Sonnenburg JL, Chen CTL, Gordon JI (2006) Genomic and metabolic studies of the impact of probiotics on a model gut symbiont and host. PLoS Biol 4:413. https://doi.org/10.1371/journal.pbio.0040413

Soufli I, Toumi R, Rafa H et al (2015) Crude extract of hydatid laminated layer from Echinococcus granulosus cyst attenuates mucosal intestinal damage and inflammatory responses in Dextran Sulfate Sodium induced colitis in mice. J Inflamm 12:19. https://doi.org/10.1186/s12950-015-0063-6

Spanogiannopoulos P, Bess EN, Carmody RN, Turnbaugh PJ (2016) The microbial pharmacists within us: a metagenomic view of xenobiotic metabolism. Nat Rev Microbiol 14:273. https://doi.org/10.1038/nrmicro.2016.17

Spiljar M, Merkler D, Trajkovski M (2017) The immune system bridges the gut microbiota with systemic energy homeostasis: focus on TLRs, mucosal barrier, and SCFAs. Front Immunol 8:1353. https://doi.org/10.3389/fimmu.2017.01353

Takahashi N, Xiao J-Z, Miyaji K et al (2004) Selection of acid tolerant bifidobacteria and evidence for a low-pH-inducible acid tolerance response in Bifidobacterium longum. J Dairy Res 71:340. https://doi.org/10.1017/s0022029904000251

Tanabe S, Kinuta Y, Saito Y (2008) Bifidobacterium infantis suppresses proinflammatory interleukin-17 production in murine splenocytes and dextran sodium sulfate-induced intestinal inflammation. Int J Mol Med 22:181–185

Tankanow RM, Ross MB, Ertel IJ et al (1990) A double-blind, placebo-controlled study of the efficacy of Lactinex in the prophylaxis of amoxicillin-induced diarrhea. DICP 24:382–384. https://doi.org/10.1177/106002809002400408

Tarahomjoo S (2012) Development of vaccine delivery vehicles based on lactic acid bacteria. Mol Biotechnol 51:183–199. https://doi.org/10.1007/s12033-011-9450-2

Taverniti V, Guglielmetti S (2011) The immunomodulatory properties of probiotic microorganisms beyond their viability (ghost probiotics: proposal of paraprobiotic concept). Genes Nutr 6:261–274. https://doi.org/10.1007/s12263-011-0218-x

Thursby E, Juge N (2017) Introduction to the human gut microbiota. Biochem J 474:1823–1836. https://doi.org/10.1042/BCJ20160510

Toumi R, Soufli I, Rafa H et al (2014) Probiotic bacteria lactobacillus and bifidobacterium attenuate inflammation in dextran sulfate sodium-induced experimental colitis in mice. Int J Immunopathol Pharmacol 27:615–627. https://doi.org/10.1177/039463201402700418

Trush EA, Poluektova EA, Beniashvilli AG et al (2020) The evolution of human probiotics: challenges and prospects. Probiotics Antimicrob Proteins. https://doi.org/10.1007/s12602-019-09628-4

Vanderpool C, Yan F, Polk BD (2008) Mechanisms of probiotic action: implications for therapeutic applications in inflammatory bowel diseases. Inflamm Bowel Dis 14:1585–1596. https://doi.org/10.1002/ibd.20525

Venturi A, Gionchetti P, Rizzello F et al (1999) Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. Aliment Pharmacol Ther 13:1103–1108. https://doi.org/10.1046/j.1365-2036.1999.00560.x

Vinolo MAR, Rodrigues HG, Hatanaka E et al (2011) Suppressive effect of short-chain fatty acids on production of proinflammatory mediators by neutrophils. J Nutr Biochem 22:849–855. https://doi.org/10.1016/j.jnutbio.2010.07.009

Waldman AJ, Balskus EP (2018) The human microbiota, infectious disease, and global health: challenges and opportunities. ACS Infect Dis 4:14–26. https://doi.org/10.1021/acsinfecdis.7b00232

Walker AW, Sanderson JD, Churcher C et al (2011) High-throughput clone library analysis of the mucosa-associated microbiota reveals dysbiosis and differences between inflamed and non-inflamed regions of the intestine in inflammatory bowel disease. BMC Microbiol 11:7. https://doi.org/10.1186/1471-2180-11-7

Wells JM, Brummer RJ, Derrien M et al (2016) Homeostasis of the gut barrier and potential biomarkers. Am J Physiol Liver Physiol 312:G171–G193. https://doi.org/10.1152/ajpgi.00048.2015

Whorwell PJ, Altringer L, Morel J et al (2006) Efficacy of an encapsulated probiotic Bifidobacterium infantis 35624 in women with irritable bowel syndrome. Am J Gastroenterol 101:1581–1590. https://doi.org/10.1111/j.1572-0241.2006.00734.x

Yan F, Polk DB (2011) Probiotics and immune health. Curr Opin Gastroenterol 27:496. https://doi.org/10.1097/MOG.0b013e32834baa4d

Zaki MH, Boyd KL, Vogel P et al (2010) The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis. Immunity 32:379–391. https://doi.org/10.1016/j.immuni.2010.03.003

Zhang C, Derrien M, Levenez F et al (2016) Ecological robustness of the gut microbiota in response to ingestion of transient food-borne microbes. ISME J 10:2235–2245. https://doi.org/10.1038/ismej.2016.13

Zheng J, Wittouck S, Salvetti E 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. Int J Syst Evol Microbiol 70:2782–2858. https://doi.org/10.1099/ijsem.0.004107

Zojaji H, Ghobakhlou M, Rajabalinia H et al (2013) The efficacy and safety of adding the probiotic Saccharomyces boulardii to standard triple therapy for eradication of H. pylori: a randomized controlled trial. Gastroenterol Hepatol 6:99

The Progressive Development of Probiotics, Prebiotics, and Synbiotics Research and Its Multipurpose Use in the Ornamental Fishery

6

Manab Kumar Saha, Manojit Bhattacharya, and Bidhan Chandra Patra

Abstract

Ornamental fish culture is the most popular hobby throughout the world, and it has a great role to stabilize the economy in developing countries. Health and nutrition are two pillars that hold the ornamental fish trade. Quality assurance program has become an essential part of the ornamental fish culture. Antimicrobial-resistant pathogens have been amplified due to excessive use of antibiotics in aquaculture over the past three decades. That is why, safe and sound substitute to antibiotics is very essential for sustainable development for the aquaculture sector after its annulment in the European Union (EU) in 2006. It is essential to study the various ecological aspects and their relationship minutely regarding the ex situ cultivation of brood stock to avoid the wild collection of the ornamental fishes. In search for alternatives of antibiotics, an eco-friendly and beneficial bacteria are used, known as prebiotics, probiotics, and synbiotics, which improve the microbial ecology of the fish gut. Recent research work shows that probiotics have no side effects on the aquatic environment (aquarium) and on the ornamental fishes; rather, they produce several essential digestive enzymes and vitamins and also amplify the admittance to minerals and microelements. Probiotics show a synbiotic benefits to the ornamental fishes as improved gastrointestinal morphology, which plays a vital responsibility in preventing and controlling pathogens, thereby promoting larval survival,

M. K. Saha (✉)
Department of Zoology, Ramananda Centenary College, Laulara, Purulia, West Bengal, India

M. Bhattacharya
Department of Zoology, Fakir Mohan University, Vyasa Vihar, Balasore, Odisha, India

B. C. Patra
Centre for Aquaculture Research, Extension and Livelihood, Department of Aquaculture Management and Technology, Vidyasagar University, Midnapore, West Bengal, India

K. K. Behera et al. (eds.), *Prebiotics, Probiotics and Nutraceuticals*,
https://doi.org/10.1007/978-981-16-8990-1_6

enhanced immunostimulants, and improved disease resistance and getting better tolerance, increased growth performance, improved body color quality, reduced stress response, reduced malformations, etc. These entire factors are positively correlated with better fish appetite, feed conversation ratio, and feed utilization as confirmed by findings of many studies. Rearing and reproduction of the ornamental fish species have notably increased and have received the attention of the hobbyists to scientists as the ornamental fishes have high economic value. Reproduction is an energy-consuming process that can be successfully performed only when adequate energy stores are available within the organisms. Present researches indicate that probiotics play a crucial role between metabolism and reproduction. To get the highest benefit from probiotics, prebiotics, and synbiotics research, it is essential to know the mechanism of action to attain a better quality of the environment and healthy ornamental fish culture practices, suggesting avoidance of pharmaceutical use.

Keywords

Functional feed · Probiotics · Prebiotics · Synbiotics · Mode of action · Sustainable ornamental fish culture

6.1 Introduction

Ornamental fish rearing, culture, and breeding offer brilliant scope, not only aesthetic gratification to the hobbyist but also self-employment and financial openings. The growth curve of ornamental fishes for the last four decades has created its prominent and strong footprint in the international trade of fisheries (Sharma et al. 2019). It is one of the major beneficial and profitable areas of nonfood fish farming activities (Ghosh et al. 2008a, b; Kumar and Gopal 2015). The global ornamental fish trade is approximated to be more than US $15 billion, and more than 2 billion live ornamental fishes are traded (Satam et al. 2018). The major share of this ornamental fish market belongs to the freshwater species (Wood 2001). As a result, this trade occupies its cover area in a sustainable economy day by day in the developing countries for employment generation and livelihoods. The demands for ornamental fishes are increased by leaps and bounds. Consequently, ornamental fish farmers or the company's culture these species on a large scale to meet the demand of the national and international hobbyists. So, intensive ornamental fish culture is the ultimate output, which accompanies numerous infections due to some pathogens. Excessive stocking density, high food input for fast growth rate, execratory product, other organic wastes, and unutilized food create a favorable environment for the explosion of some pathogenic bacteria (Austin et al. 1995; Austin and Austin 2007). Sudden mass mortality of their stock due to the proliferation of infectious microbial community which occurs both in the aquatic environment and in the fish gut. This creates a negative impact on culture and economy as well (Paperna 1991; Jeney and Jeney 1995; Austin and Austin 1999; Moriarty 1999; Jana and Jana 2003). To solve these problems, some common and popular methods have been applied by the farmers such as lime applications, frequent water exchange, and aeration which are costly and time-consuming (Serrano 2005; Ladoni et al. 2010). Subsequently, the farmers are emphasizing on the identification and elimination of infectious pathogens from the culture media for the sustainable growth of the industry. Till now, the random use of chemotherapy and antibiotics are well accepted by the farmers due to its quick effect and easy availability. The excessive use of antibiotics has a high possibility of the development of antibiotic-resistant bacteria and has permanent adverse effects on the aquatic ecosystem (Serrano 2005; Steenbergen et al. 2015). Growth, body color, normal biological activity, and production efficiency ultimately give profitability of the ornamental fisheries (Baron et al. 2008). This is solely dependent upon the fish's health, which is directly dependent on the quality of rearing water and supplied food. In this perspective, probiotics are gradually more popular, because they help in increasing the fish production and inhibiting the growth of virulent pathogenic infection in the same aquatic habitat (de Kinkelin and Michel 1992; Gatesoupe 1999; Moriarty 1999; Skjermo and Vadstein 1999; Uma et al. 1999; Sharma and Bhukar 2000; Sze 2000; Jana and Jana 2003). In the year 1908, Russian scientist Elie Metchnikoff gave the concept that intestinal flora can be altered – "the infectious microbes restored with advantageous" (Dubey et al. 2019). The word probiotic is coined by a combination of two words, the Latin preposition *pro*, which means "for," and the Greek word *biotic* meaning "bios" or "life." According to the World Health Organization, probiotics are distinct as "live microorganisms which, when applied in sufficient amounts, give out a health care on the host" (Ghosh et al. 2008a, b; Vinderola et al. 2011; Abildgaard et al. 2018). Most commonly and widely feeds employed as nutrient supplements for brood stock of ornamental fish are herbal products, nutrient mixtures, hormones, antibiotics, and live feeds, but these feed supplements can cause inhibition of the beneficial microbiota in the gut of the breeders (Fernando et al. 1991). In this circumstance, the breeders have a high chance to infect the opportunistic pathogens. Probiotics can be used as feed supplements to restore the beneficial bacteria in the gut of the host animal (Fuller 1989). In fish, the application of probiotics to enhance the immunity and bio-growth parameters has been well established (Ghosh et al. 2008a, b). Probiotics have been used to stimulate appetite, detoxify the diet by digesting the toxic components, and develop the quality of nutrition by the production of vitamins, protein, fatty acids, and carotenoids, etc. Probiotics have also important role in fertilization, fecundity, hatching, and larval development (Izquierdo et al. 2001; Mazorra et al. 2003; Afzal Khan et al. 2005; Aly et al. 2008). So, probiotics have the capability to enhance fish health by reducing pathogen load and improving water quality (Fast and Menasveta 2000; Gomez-Gil et al. 2000; Jana and Jana 2003; Hong et al. 2005; Lalloo et al. 2007). Still, the primary required condition of biological agents is "colonization" for survival in the environment and increasing welfare (Makridis et al. 2000; Gross et al. 2003; Ziaei-Nejad et al. 2006; Kechagia et al. 2013; Steenbergen et al. 2015). Synbiotics may be identified as combined dietary supplements of probiotics and prebiotics (Nekoubin et al. 2012). In this discussion, we have summarized the evolution and present status of probiotics, prebiotics, and synbiotics research and their multipurpose use in the ornamental fishery (Lulijwa et al. 2020).

6.2 Evolution of the Concept of Probiotics

The most accepted definition of prebiotics was coincided by Gibson et al. (2004); they alter "pro" for "pre," which means "before" or "for." The term probiotics refer to "a supplementary food component that has a beneficial effect on hosts' growth and activity of selective microflora in the colon" (Table 6.1).

Some common terms and there definitions:

Bacteriophages: Viruses that contaminate bacterial cells

Microbiota: Each and every one commensal, pathogenic, and symbiotic microorganism sharing a distinct niche; here, it refers to the intestinal ecosystem (Pérez-Sánchez et al. 2018).

Phytobiotics: Natural bioactive plant-derived chemicals mixed with the diet to get better nutrition and health in cultivated animals and humans (Cheng et al. 2014).

Postbiotics: Metabolic by-products or nonviable bacterial products of probiotic microorganisms which are biologically active and show different function in the host (Patel and Denning 2013).

Prebiotics: Selective fermented components that stimulate the growth and some immune-related activity in the gastrointestinal microbiota that confers benefits upon host health (Gibson et al. 2004).

Probiotics: The presence of selective beneficial microorganisms generally present in ample amounts that shows several health benefits on the host (Reid et al. 2003).

Synbiotics: Effective and selective composition of probiotics and prebiotics, which can give more stable or synergistic effects (Huynh et al. 2017).

6.3 Probiotics for Sustainable Aquaculture

Aquaculture, particularly the fisheries, is the prime potential sector. This sector has a huge responsibility to check food security (De et al. 2014; Koehn et al. 2017). To accomplish the massive demand, harmful chemicals are used immediately to fulfill the demand of the people. This phenomenon has a harmful impact both on the aquatic environment and on the target aquatic species (Dawood and Koshio 2016; Hoseinifar et al. 2016). As a result, the probability of disease outbreaks increased especially in the case of fisheries. To control this situation, antibiotics are widely used to control infection in aquaculture (Zorriehzahra et al. 2016; Dawood et al. 2018, 2019; Goya et al. 2020). The main part that determines the trade and success of the ornamental fish industry is the health of the fishes. The fish health is directly dependent on their physicochemical environment of rearing water quality and food supplement. The random use of antibiotics and chemicals led to developing the drug-resistant strains of pathogenic microorganisms. Subsequently, antimicrobial-resistant pathogens have been greater than before due to using antibiotics in

Table 6.1 The history of the term probiotics (Schrezenmeir and de Vrese 2001)

Sl. no.	Observation	Concept
1.	Metchnikoff and Chalmers-Mitchell (1908)	Beneficial microbes that can modify the gut microbiota and restore useful microbes by replacing harmful microbes
2.	Lilly and Stillwell (1965)	Substances secreted by one group of microorganism which enhance the expansion of another group
3.	Sperti (1971)	Probiotics were identified as tissue extracts that stimulate microbial growth
4.	Parker (1974)	Organisms and substances which keep up the intestinal microbial stability
5.	Fuller (1989)	A live microbial feed complement which beneficially affects the host animal by getting better its intestinal microbial equilibrium
6.	Havenaar and Huis (1992)	A viable single culture or multiculture of microorganisms which is useful to animal or man, constructively affects the host by getting improved qualities of the indigenous microflora
7.	Salminen (1996)	A live microbial cultured dairy invention which constructively influences the health and nutrition of the host
8.	Schaafsma (1996)	Oral probiotics are living microorganisms which upon intake in convinced numbers apply health effects ahead of intrinsic basic nutrition
9.	Molin (2001)	Cultured dairy products and microbial cultures to be probiotic. Without a doubt, the matrix of a product may influence the activity of microbes and consequently the continued existence and effect of the microbes and thus deserves consideration
10.	de Vrese et al. (2001)	Evidence for health effects away from nutritional importance of such products that is immunomodulating and carcinogenic effects have been exerted by cell wall components of lactobacilli and bifidobacteria
11.	Reid et al. (2003)	Yogurt containing usual cultures (*Lactobacillus delbrüecki* and *Streptococcus thermophilus*) as these cultures may recompense for lactase deficiency in lactose maldigestion

aquaculture (Ouwehand et al. 2014). That is why, an eco-friendly, nonconventional, effective, safe, and sound alternative to antibiotics is essential for sustainable development like probiotics and with the ability to improve nutritional efficiency, general health status, and development in the aquaculture sector (Dawood and Koshio 2016; Van Doan et al. 2020; Xue et al. 2017). The resentment mechanism, based on the exclusion of the pathogen by the beneficial bacterial population and the dietary benefits of a bioaugmentor (Gatesoupe 1999), has been regarded as being of great significance by most authors. It is very important to know the mechanisms of action of probiotics, mainly the intestinal ecosystem. Probiotics characterize as “a live microbial feed complement which constructively affects the host species" by recovering the equilibrium of gut microflora. So, probiotics are renowned as "live microorganisms when added in adequate amounts provide health support on the host" (Food and Agriculture Organization of the United Nations and World Health Organization 2001). For the most excellent result, probiotics have to be used from the larval stage of fish and shellfish species. The larval forms are essentially released into the aquatic environment near the beginning of ontogenetic phase (Kapareiko et al. 2011; Arıg et al. 2013). Probiotics with immunomodulation chattels can boost the efficiency of an organism's response to stress like changes of ion concentration, dissolved oxygen, temperature, PH fluctuation, etc. (Magnadottir 2010). Probiotics are engaged in aquaculture and have numerous forms including bacterial cells (*Lactobacillus* sp., *Bacillus* sp., *Lactococcus* sp., *Enterococcus* sp., *Weissella* sp., *Micrococcus* sp., *Carnobacterium* sp., and *Streptococcus* sp.), yeasts (*Saccharomyces* sp., *Debaryomyces* sp., and *Phaffia* sp.), and microalgae (*Tetraselmis* sp.) (Martínez Cruz et al. 2012).

6.4 Probiotics for Ornamental Fish Culture

Tropical ornamental fish have high demand in the international fish market. The live-bearing group of ornamental fishes is largely popular among the fish hobbyists and entrepreneurs. The live-bearing ornamental fishes are brilliantly colored, agree to take all types of food, and profusely produce living free-swimming young ones. Among live-bearers, *Poecilia sphenops* (Valenciennes), *Poecilia reticulata* (Peters), *Xiphophorus maculatus* (Gunther), and *Xiphophorus hellerii* (Heckel) are the most significant and well-known ones. In ornamental fish, the use of probiotics for enhancing better disease resistance capability and growth parameters has been well accepted.

According to Dharmaraj and Dhevendaran (2010), the probiotic fish feed showed noteworthy improvement in length compared with the control fish feed. Probiotic microorganisms produce vitamins and macromolecules which promote animal nutrition (Fig. 6.1).

A significant increment in the growth and length of the ornamental fish *Xiphophorus hellerii* was reported when they were fed with *Streptomyces* as probiotic. Apart from that, some common organisms in probiotic products are *Lactobacillus acidophilus*, *L. bulgaricus*, *L. plantarum*, *Aspergillus oryzae*, *Bacillus* sp., *Streptococcus lactis*, *Saccharomyces cerevisiae*, and *Bifidobacterium bifidum*. These have numerous advantages like (a) the production of heat- and desiccation-resistant spores, (b) the formation of antimicrobial substances, and (c) the degradation of macromolecules, such as protein, the starch in the pond culture. Among live-bearers, *Poecilia sphenops* (Valenciennes), *Poecilia reticulata* (Peters), *Xiphophorus maculatus* (Gunther), and *Xiphophorus hellerii* (Heckel) are the most familiar and imperative ones. Experimentally, it is established that an isolated probiotic, *B. subtilis*, when added as a food supplement, improved the GSI (gonadosomatic index), fecundity, and fry survival and morphometric characteristics of fry of all the

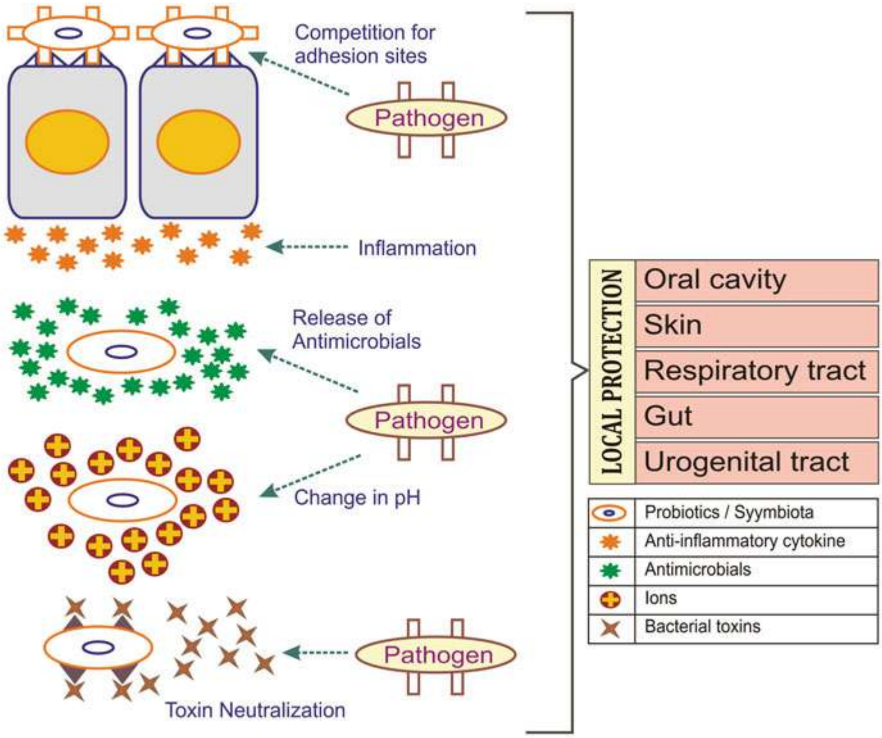


Fig. 6.1 Schematic diagram of local protection action of probiotics against pathogens

four species. Feed supplementation with probiotics gives a significant result related to the gonadosomatic index, fecundity, and health in all the four species of fish. The survival rate was also significantly higher in the fish fry, fed with the probiotic feeds. Ghosh et al. (2007) showed that female ornamental live-bearers, during their reproductive stages, benefit from the addition of probiotics in the diet (Fig. 6.2).

Probiotic supplements are capable of fulfilling the growth and reproductive deficiencies. Schrezenmeir and de Vrese (2001) defined probiotics as "by-product of distinct viable microorganisms in adequate numbers, which change the microflora in gut ecology and in this way they show beneficial health effects in this host." The nutritional benefits of probiotic bacteria take account the production of vital digestive enzymes, production of vitamin, availability of minerals and trace elements, etc. The green terror fish shows a better innate immune system and growth rate when they are fed with probiotic *Pediococcus acidilactici* as an effective dietary additive (Neissi et al. 2013). Probiotic bacteria are recognized as immune stimuli through a nonspecified defense system that could enhance the resistance of the host. This species (*P. acidilactici*) could tolerate a broad range of acidity. Divya et al. (2012) conducted a study to measure the consequence of probiotic bacteria such as *Bacillus mesenteric*, *Bacillus coagulans*, and *Bifidobacterium infantis* in the intestine of

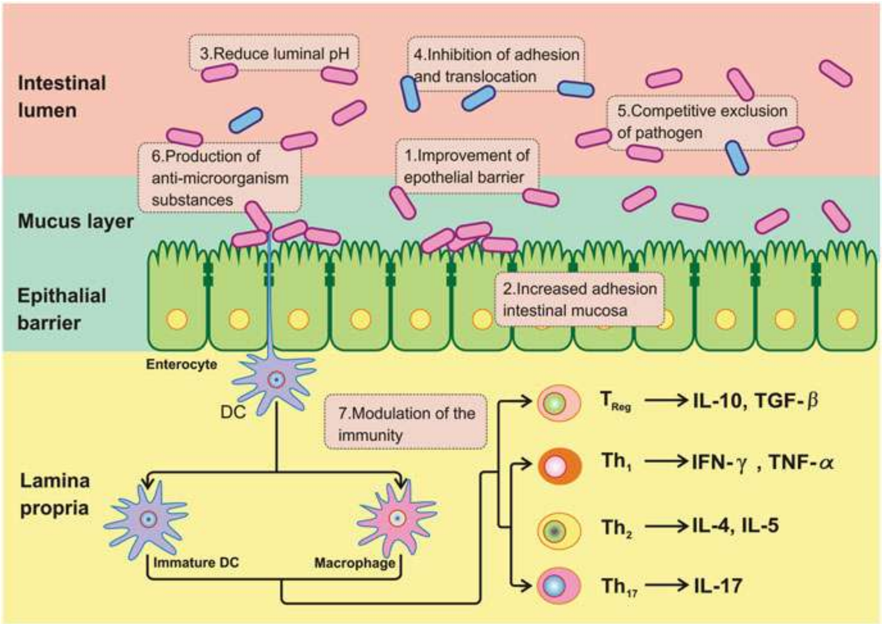


Fig. 6.2 General mechanism of action of probiotics within ornamental fish gut. *IL* interleukin, *IFN* interferon, *TGF* transforming growth factor, *DC* dendritic cell. This figure has been adapted from Zhang et al. (2017)

freshwater ornamental fish *Puntius conchonius*. The results showed that the probiotic bacteria were considerably established in the gut of *P. conchonius* and significantly inhibit the pathogenic population in the gut of the fish. Copepods play a significant role in the development of larvae, postlarvae, and juveniles of ornamental fishes, and they are also able to accumulate beneficial bacteria. Fish growth and survival, water quality parameters, and bacterial population of water were assessed (Ghosh et al. 2008a, b). *Bacillus subtilis* was isolated from the gut of *Cirrhinus mrigala* and was mixed into the rearing water of *Poecilia sphenops* (Valenciennes), *Xiphophorus hellerii* (Heckel), *Xiphophorus maculatus* (Gunther), and *Poecilia reticulata* (Peters) with different concentrations to observe the effect. The results showed that the bacterial cells present in the rearing water resulted in better survival and a quicker growth rate (greater length and weight increments of the live-bearers). The consequence of feeding a probiotic bacterium *Lactobacillus* sp. with bioencapsulated live food organism *Artemia* sp. shows increased growth and production of ornamental fishes such as *Carassius auratus* and *Xiphophorus hellerii*. The inoculation of *Lactobacillus* sp. into the ornamental fish rearing systems has an effect on the water quality with significant changes in dissolved organic matter, chemical oxygen demand, and phosphate phosphorus of the rearing water. The bioencapsulated probiotic feed gives a significant improvement in food conversion ratio, wet weight gain, and specific growth rate of *C. auratus* and *X. hellerii* fry. This

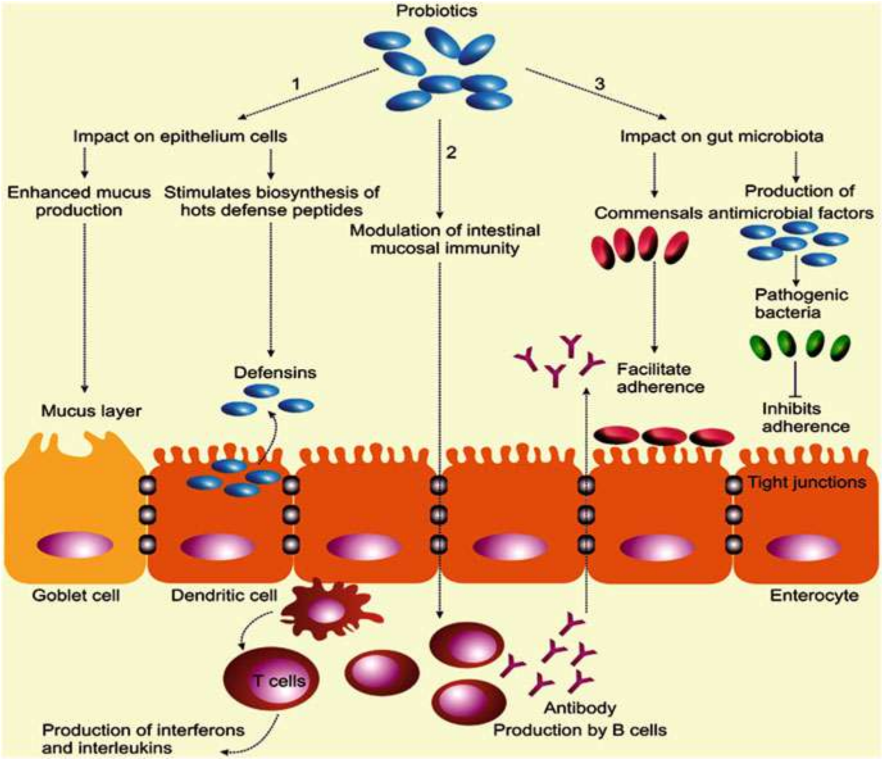


Fig. 6.3 Major benefits of probiotics for maintaining the optimum aquatic ecology for ornamental fish culture. This figure has been adapted from Khurshid et al. (2015)

technology is eco-friendly and could be adopted effectively in the commercial production of ornamental fishes (Nandi et al. 2009). The supremacy of Gram-positive antagonistic bacteria in freshwater fishes implies that these bacteria could be helpful as probiotics and/or biocontrol component in freshwater ornamental fish rearing. A search for beneficial antagonistic bacteria like *Asporogenous* and aerobic spore-forming *Bacillus* sp. was the prevailing antagonist groups followed by *Micrococcus*, *Lactobacillus*, *Lactococcus*, and *Streptococcus* from the fish gut of ornamental fishes and in the rearing water (Fig. 6.3).

Their use as probiotics in ornamental fish culture was attempted. The latent probiotic strains, *Bacillus* sp. and *Lactobacillus* sp., from the fish gut were accomplished with different substrates and conferred security to *Carassius auratus* and *Xiphophorus hellerii*. *Bacillus* sp. survived well both in pelleted feed and water, while the *Lactobacillus* sp. is unable to do so (Abraham et al. 2004). Probiotic selection principally depends on methods of production and processing, biosafety considerations, method of administration, and the location in the body where the microorganisms are normal to be active. The indigenous fish gut bacteria provided an advantage to the ornamental fishes in terms of survival and adherence to different substrates and thus satisfied the chief needs of being effective probiotics (Salminen 1996). *Lactobacillus rhamnosus* bacteria were supplied as probiotic to clownfish (*Amphiprion ocellaris*) larvae from the very beginning. Results showed double body weight gain in both clownfish when *Lactobacillus rhamnosus* was abounding with live prey in the rearing water. There was considerably increased gene expression related to growth (myostatin, insulin-like, vitamin D receptor, peroxisome proliferators-activated receptors, and retinoic acid receptor). Furthermore, probiotics also minimize stress by lowering the glucocorticoid receptor gene expression. The result suggests a strong effect of lactic acid bacteria on the development of larval clownfish (Avella et al. 2010). Probiotic bacteria such as *Lactobacillus rhamnosus*, *Bacillus mesentericus*, *Bacillus coagulans*, *Bifidobacterium longum*, and *Bifidobacterium infantis* are inhabiting the intestine of the freshwater ornamental fish, *Brachydanio rerio*. Experimentally it is proved that the beneficial bacteria were significantly established in the intestine of *B. rerio* fish. To apply their advantageous effects, probiotics must resist the acidity of the stomach (Tuomola et al. 2001). A study found that feeding probiotics with *Artemia franciscana* nauplii, to fish *Brachydanio rerio* larvae, showed a high survival rate. The management of probiotics considerably altered the quantity of gut microflora of zebra fish larvae. Hoseinifar et al. (2015) observed the special effects of different concentrations of dietary probiotic *Lactobacillus acidophilus* as a feed supplement on gut microbiota, cutaneous mucous immune parameters and salinity stress resistance, and also growth performance of black swordtail (*Xiphophorus hellerii*). Microbiological assessments have shown that feeding with a probiotic-supplemented diet extremely increased entirely autochthonous bacteria and autochthonous lactic acid bacteria levels. Feed supplementation probiotic *L. acidophilus* when fed to swordtail shows significant growth performance, mucosal immune response, stress resistance, and also establishment of intestinal microbiota (Hoseinifar et al. 2015) (Table 6.2).

The health effects recognized in the use of probiotics are numerous. The following observations (Schrezenmeir and de Vrese 2001) are well accepted:

1. Boost of humoral and cellular immunity
2. Minor occurrence and duration of diarrhea linked with antibiotics, chemotherapy, rotavirus infection, and, to a slighter amount, traveler's diarrhea
3. Reduction of allergic symptoms
4. Decline of helicobacter pylori infection
5. Positive effects on mineral metabolism, chiefly bone density, and stability

6.5 Relation Among Probiotics, Prebiotics, Synbiotics, or Postbiotics and Their Applications in Ornamental Fish Culture

Fish is exposed to an aquatic environment like other aquatic animals, where different types of microorganisms are present. Each microorganism shares a distinct niche (gut ecosystem) which is recognized as the microbiota. The microbiota is

Table 6.2 Advantages and disadvantages of probiotics (García-Arroyo et al. 2018; Hosain and Liangyi 2020)

Sl. no.	Advantages	Limitations
1.	Result in better growth and health condition	Some degree of defense with limited pathogens
2.	Supply nutrition for aquatic animals	The use in the gut for fish is studied but no more results found in other organs
3.	Avoid colonization of pathogenic microbes and reduce the chances of infection	Marketing agreement is very intricate
4.	Reduce the amount of blue-green algae load and clean the bottom of the pond	Misguidance may oppose the new pathogens and will be unsafe for the human being
5.	Obstruction of pathogens in the gut or elsewhere	Unacceptable function in host sometime may be negatively treated
6.	Improve water quality and ensure a friendly environment	Mislabeling and mishandling may cause an adverse condition for the environment
7.	Target-oriented, whereby protecting the host microbiota	Prevent the transfer of virulence or antibiotic resistance gene
8.	Water exchange essential less that reduces water pumping costs	Advance technology necessary to arrange probiotics which are the main challenge
9.	Improved digestibility and microbial adjustment	Probiotics work gradually than antibiotics
10.	Reduced green vibrio counts and ammonia level in water ensuring healthier production and increased profits	No issue
11.	Host immune response stimulation to disease	It cannot reach and preserve itself in the location where the consequence is to be applied

significantly engaged in an extensive range of metabolic activities in the host body (Galindo-Villegas et al. 2012). The gut microbiota regulates three main aspects: (a) dietary assistance, (b) removal and/or protection against harmful microbes (pathogens), and (c) simulation control of the immune responses. Probiotics control all the favorable metabolic activity for sustainable growth (Pérez et al. 2010). Probiotics do check the growth of pathogens by suppression of virulence gene expression, secretion of antimicrobial chemicals (bacteriocins, antibiotics, hydrogen peroxide, lytic enzymes, fatty acids, etc.), antagonism for nutrient resource, and/or disruption of quorum sensing (Pérez-Sánchez et al. 2011; Lauzon et al. 2014) (Fig. 6.4).

Molecular research indicates that bacteriocins are a group of vital chemicals that play a significant role in chemical signaling known as quorum sensing. Quorum sensing is nothing but a cell signaling process (Auto Inducers) that includes recognition, fabrication, and response to an extracellular signal, e.g., biofilm formation (Ventura et al. 2015). Probiotics have greater affinities to glycol conjugate receptors present on the surface of the intestinal epithelial cells compared to the pathogenic

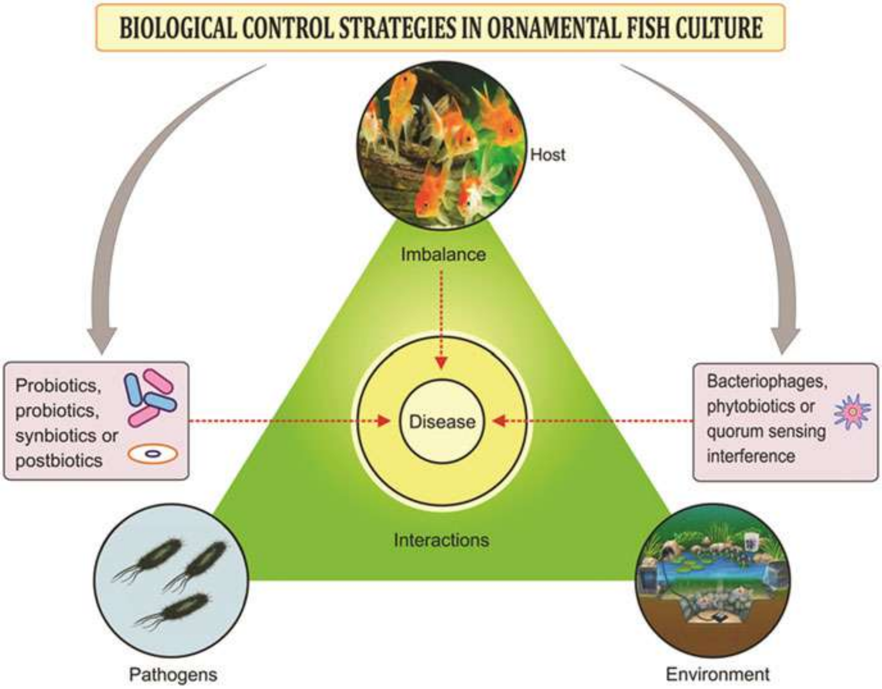


Fig. 6.4 Interactions between "environment-pathogen-host probiotics" and a hypothetical diagram of protection by creating an antagonistic environment for pathogens. This figure has been adapted from Defoirdt et al. (2007)

bacteria by producing more mucus, short-chain fatty acids, cytokines, etc. (Austin and Sharifuzzaman 2017).

As a result, prebiotics creates a favorable microenvironment for the beneficial bacteria that were colonized and grow within the intestine of fishes. Furthermore, prebiotics can generate some chemical signals through their selective metabolism like production of pro- and anti-inflammatory cytokines, which are crucial chemical messengers involved in the regulation, activation, growth, and differentiation of immune cells (Pérez-Sánchez et al. 2018).

6.6 Concluding Remarks

Aquaculture holds a vital role along with the fastest-growing sectors worldwide. Ornamental fisheries are becoming the largest growing and upcoming sector in terms of job opportunities and income-generating sectors. Due to the high demand of these ornamental fishes, the industries have huge production pressure. As a result, infections and disease outbreaks affect the economic development of the country. It leads to an increase in the abrupt use of antibiotics to get an immediate result and produce antibiotic-resistant microorganisms as a by-product. Antibiotic resistance is a solemn warning to global public health. Consequently, proper strategies have to be established to check the spread of antibiotic resistance. Probiotics, prebiotics, synbiotics, and bacteriophage intrusion may be measured as eco-friendly strategies for disease control in ornamental fish culture. Probiotics, prebiotics, and synbiotics are nothing but sustainable and useful source of beneficial microbes (Pérez-Sánchez et al. 2018). There should be a strong interaction and collaboration among fish sector owners, producers, academicians, and scientists to point out the particular aspects of beneficial microbes ensuring the prevention of antibacterial, antifungal, antiviral, and anti-pathogenic activity. Probiotics, Prebiotics, and Synbiotics have better growth performance, antistress molecule production, immune-boosting capabilities, and disruption of QS (quorum sensing) as a new anti-infective strategy to promote production and economic growth of the ornamental fish industry. For that reason, it is compulsory to carry out more research to determine optimum inclusion levels for host-associated probiotics. For better understanding of the relation and mechanism of host-associated probiotics, it is essential to study the transcriptome and proteome profiling of gut microbiota, intestinal epithelium, interactions between gut microbes, gut immune system, antioxidant status, lipid level of hosts, etc. (Zorriehzahra et al. 2016). Furthermore, information regarding auto-aggregation capacity, cell surface hydrophobicity, development of color in fish, and different sources of probiotics need to be investigated. There is no evidence of the negative impacts of probiotics on ornamental fish culture. (Hosain and Liangyi 2020). Besides, the gnotobiotic approaches should be included to understand the molecular interaction and function of gut microbiota on various biological processes of the host to identify whether they are viable or nonviable probiotics (Van Doan et al. 2020). From environmental safety point of view, probiotics should be workable on bulky production. It should also be taken into consideration that probiotics must be available at a low cost for ornamental fish farmers. It will insist that farmers use this eco-friendly feed supplement to maintain fish health and reduce disease-causing microorganisms in the ornamental fisheries sector.

References

Abildgaard A, Elfving B, Hokland M, Wegener G, Lund S (2018) The microbial metabolite indole-3-propionic acid improves glucose metabolism in rats, but does not affect behaviour. Arch Physiol Biochem 124(4):306–312

Abraham TJ, Sasmal D, Banerjee T (2004) Bacterial flora associated with diseased fish and their antibiogram. J Indian Fish Assoc 31:177–180

Afzal Khan M, Jafri AK, Chadha NK (2005) Effects of varying dietary protein levels on growth, reproductive performance, body and egg composition of rohu, Labeo rohita (Hamilton). Aquac Nutr 1:11–17

Aly SM, Abd-El-Rahman AM, John G, Mohamed MF (2008) Characterization of some bacteria isolated from Oreochromis niloticus and their potential use as probiotics. Aquaculture 277(1-2): 1–6

Arıg N, Suzer C, Gökvardar A, Başaran F, Çoban D, Yıldırım Ş, Kamacı HO, Fırat K, Saka Ş (2013) Effects of probiotic (*Bacillus sp.*) supplementation during larval development of Gilthead sea bream (*Sparus aurata L.*). Turk J Fish Aquat Sci 13(3):407–414

Austin B, Austin DA (1999) Bacterial fish pathogens, 3rd edn. Springer Praxis, Chichester

Austin B, Austin DA (2007) Bacterial fish pathogens: diseases of farmed and wild fish, 3rd edn. Springer-Praxis, Godalming, p 552

Austin B, Sharifuzzaman SM (2017) Probiotics for disease control in aquaculture. Diagnosis and Control of Diseases of Fish and Shellfish, pp 189–222

Austin B, Stuckey LF, Robertson PA, Effendi I, Griffith DR (1995) A probiotic strain of Vibrio alginolyticus effective in reducing diseases caused by Aeromonas salmonicida, Vibrio anguillarum and Vibrio ordalii. J Fish Dis 18(1):93–96

Avella MA, Olivotto I, Silvi S, Place AR, Carnevali O (2010) Effect of dietary probiotics on clownfish: a molecular approach to define how lactic acid bacteria modulate development in a marine fish. Am J Physiol 298(2):R359–R371

Baron M, Davies S, Alexander L, Snellgrove D, Sloman KA (2008) The effect of dietary pigments on the coloration and behaviour of flame-red dwarf gourami, Colisa lalia. Anim Behav 75(3): 1041–1051

Cheng G, Hao H, Xie S, Wang X, Dai M, Huang L, Yuan Z (2014) Antibiotic alternatives: the substitution of antibiotics in animal husbandry? Front Microbiol 5:217

Dawood MA, Koshio S (2016) Recent advances in the role of probiotics and prebiotics in carp aquaculture: a review. Aquaculture 454:243–251

Dawood MA, Koshio S, Esteban MÁ (2018) Beneficial roles of feed additives as immunostimulants in aquaculture: a review. Rev Aquac 10(4):950–974

Dawood MA, Koshio S, Abdel-Daim MM, Van Doan H (2019) Probiotic application for sustainable aquaculture. Rev Aquac 11(3):907–924

de Kinkelin P, Michel C (1992) The use of drugs in aquaculture. Info Fish Int 4:45–49

De BC, Meena DK, Behera BK, Das P, Mohapatra PD, Sharma AP (2014) Probiotics in fish and shellfish culture: immunomodulatory and ecophysiological responses. Fish Physiol Biochem 40(3):921–971

Defoirdt T, Boon N, Sorgeloos P, Verstraete W, Bossier P (2007) Alternatives to antibiotics to control bacterial infections: luminescent vibriosis in aquaculture as an example. Trends Biotechnol 25(10):472–479

Dharmaraj S, Dhevendaran K (2010) Evaluation of Streptomyces as a probiotic feed for the growth of ornamental fish Xiphophorus hellerii. Food Technol Biotechnol 48(4):497–504

Divya KR, Isamma A, Ramasubramanian V, Sureshkumar S, Arunjith TS (2012) Colonization of probiotic bacteria and its impact on ornamental fish Puntius conchonius. J Environ Biol 33(3): 551

Dubey V, Mishra AK, Ghosh AR, Mandal BK (2019) Probiotic Pediococcus pentosaceus GS 4 shields brush border membrane and alleviates liver toxicity imposed by chronic cadmium exposure in Swiss albino mice. J Appl Microbiol 126(4):1233–1244

Fast AW, Menasveta P (2000) Some recent issues and innovations in marine shrimp pond culture. Rev Fish Sci 8(3):151–233

Fernando AA, Phang VP, Chan SY (1991) Diets and feeding regimes of poeciliid fishes in Singapore. Science 4:99–107

Food and Agriculture Organization of the United Nations and World Health Organization (2001). https://www.fao.org/. Cited 2005 September 8

Fuller R (1989) Probiotics in man and animals. J Appl Bacteriol 66(5):365–378

Galindo-Villegas J, García-Moreno D, de Oliveira S, Meseguer J, Mulero V (2012) Regulation of immunity and disease resistance by commensal microbes and chromatin modifications during zebrafish development. Proc Natl Acad Sci 109(39):2605–2614

García-Arroyo FE, Gonzaga G, Muñoz-Jiménez I, Blas-Marron MG, Silverio O, Tapia E, Soto V, Ranganathan N, Ranganathan P, Vyas U, Irvin A (2018) Probiotic supplements prevented oxonic acid-induced hyperuricemia and renal damage. PLoS One 13(8):e0202901

Gatesoupe FJ (1999) The use of probiotics in aquaculture. Aquaculture 180(1-2):147–165
Ghosh S, Sinha A, Sahu C (2007) Effect of probiotic on reproductive performance in female livebearing ornamental fish. Aquac Res 38(5):518–526
Ghosh S, Sinha A, Sahu C (2008a) Bioaugmentation in the growth and water quality of livebearing ornamental fishes. Aquac Int 16(5):393–403
Ghosh S, Sinha A, Sahu C (2008b) Dietary probiotic supplementation in growth and health of livebearing ornamental fishes. Aquac Nutr 14(4):289–299
Gibson GR, Probert HM, Van Loo J, Rastall RA, Roberfroid MB (2004) Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr Res Rev 17(2):259–275
Gomez-Gil B, Roque A, Turnbull JF (2000) The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. Aquaculture 191(1-3):259–270
Goya ME, Xue F, Sampedro-Torres-Quevedo C, Arnaouteli S, Riquelme-Dominguez L, Romanowski A, Brydon J, Ball KL, Stanley-Wall NR, Doitsidou M (2020) Probiotic Bacillus subtilis protects against α-synuclein aggregation in C. elegans. Cell Rep 30(2):367–380
Gross A, Nemirovsky A, Zilberg D, Khaimov A, Brenner A, Snir E, Ronen Z, Nejidat A (2003) Soil nitrifying enrichments as biofilter starters in intensive recirculating saline water aquaculture. Aquaculture 223(1-4):51–62
Havenaar R, Huis JH (1992) Probiotics: a general view. In: The lactic acid bacteria, vol 1. Springer, Boston, pp 151–170
Hong HA, Duc LH, Cutting SM (2005) The use of bacterial spore formers as probiotics. FEMS Microbiol Rev 29(4):813–835
Hosain MA, Liangyi X (2020) Impacts of probiotics on feeding technology and its application in aquaculture. J Aquac 3(1):174–185
Hoseinifar SH, Roosta Z, Hajimoradloo A, Vakili F (2015) The effects of Lactobacillus acidophilus as feed supplement on skin mucosal immune parameters, intestinal microbiota, stress resistance and growth performance of black swordtail (Xiphophorus hellerii). Fish Shellfish Immunol 42(2):533–538
Hoseinifar SH, Ringo E, Shenavar Masouleh A, Esteban MÁ (2016) Probiotic, prebiotic and synbiotic supplements in sturgeon aquaculture: a review. Rev Aquac 8(1):89–102
Huynh TG, Shiu YL, Nguyen TP, Truong QP, Chen JC, Liu CH (2017) Current applications, selection, and possible mechanisms of actions of synbiotics in improving the growth and health status in aquaculture: a review. Fish Shellfish Immunol 64:367–382
Izquierdo MS, Fernandez-Palacios H, Tacon AG (2001) Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 197(1-4):25–42
Jana BB, Jana S (2003) The potential and sustainability of aquaculture in India. J Appl Aquac 3-4: 283–316
Jeney Z, Jeney G (1995) Recent achievements in studies on diseases of common carp (Cyprinus carpio L.). Aquaculture 129(1-4):397–420
Kapareiko D, Lim HJ, Schott EJ, Hanif A, Wikfors GH (2011) Isolation and evaluation of new probiotic bacteria for use in shellfish hatcheries: II. Effects of a Vibrio sp. probiotic candidate upon survival of oyster larvae (Crassostrea virginica) in pilot-scale trials. J Shellfish Res 3:617–625
Kechagia M, Basoulis D, Konstantopoulou S, Dimitriadi D, Gyftopoulou K, Skarmoutsou N, Fakiri EM (2013) Health benefits of probiotics: a review. ISRN Nutr 2013:481651
Khurshid M, Aslam B, Nisar MA, Akbar R, Rahman H, Khan AA, Rasool MH (2015) Bacterial munch for infants: potential pediatric therapeutic interventions of probiotics. Future Microbiol 10(11):1881–1895
Koehn JZ, Allison EH, Franz N, Wiegers ES (2017) How can the oceans help feed 9 billion people? In: Conservation for the anthropocene. Ocean Academic Press, Cambridge, pp 65–88
Kumar BL, Gopal DS (2015) Effective role of indigenous microorganisms for sustainable environment. Biotech 5(6):867–876
Ladoni M, Bahrami HA, Alavipanah SK, Norouzi AA (2010) Estimating soil organic carbon from soil reflectance: a review. Precis Agric 11(1):82–99

Lalloo R, Ramchuran S, Ramduth D, Gorgens J, Gardiner N (2007) Isolation and selection of Bacillus spp. as potential biological agents for enhancement of water quality in culture of ornamental fish. J Appl Microbiol 103(5):1471–1479

Lauzon HL, Pérez-Sánchez T, Merrifield DL, Ringo E, Balcázar JL (2014) Probiotic applications in cold water fish species. Aquac Nutr 20:223–252

Lilly DM, Stillwell RH (1965) Probiotics: growth-promoting factors produced by microorganisms. Science 147(3659):747–748

Lulijwa R, Rupia EJ, Alfaro AC (2020) Antibiotic use in aquaculture, policies and regulation, health and environmental risks: a review of the top 15 major producers. Rev Aquac 12(2): 640–663

Magnadottir B (2010) Immunological control of fish diseases. Mar Biotechnol 12(4):361–379

Makridis P, Fjellheim AJ, Skjermo J, Vadstein O (2000) Control of the bacterial flora of Brachionus plicatilis and Artemia franciscana by incubation in bacterial suspensions. Aquaculture 185(3-4): 207–218

Martínez Cruz P, Ibáñez AL, Monroy Hermosillo OA, Ramírez Saad HC (2012) Use of probiotics in aquaculture. ISRN Microbiol 2012:916845

Mazorra C, Bruce M, Bell JG, Davie A, Alorend E, Jordan N, Rees J, Papanikos N, Porter M, Bromage N (2003) Dietary lipid enhancement of broodstock reproductive performance and egg and larval quality in Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture 227(1-4):21–33

Metchnikoff E, Chalmers-Mitchell P (1908) The prolongation of life. Optimistic studies. GP Putnam's Sons, New York

Molin G (2001) Probiotics in foods not containing milk or milk constituents, with special reference to Lactobacillus plantarum 299v. Am J Clin Nutr 73(2):380–385

Moriarty DJ (1999) Disease control in shrimp aquaculture with probiotic bacteria. In: Proceedings of the 8th international symposium on microbial ecology, Halifax. Atlantic Canada Society for Microbial Ecology. pp 237–243

Nandi A, Rout SK, Dasgupta A, Abraham TJ (2009) Water quality characteristics in controlled production of ornamental fishes as influenced by feeding a probiotic bacterium, *Lactobacillus* sp. bioencapsulated in *Artemia* sp. Indian J Fish 56(4):283–286

Neissi A, Rafiee G, Nematollahi M, Safari O (2013) The effect of Pediococcus acidilactici bacteria used as probiotic supplement on the growth and non-specific immune responses of green terror, Aequidens rivulatus. Fish Shellfish Immunol 35(6):1976–1980

Nekoubin H, Gharedashi E, Imanpour MR, Asgharimoghadam A (2012) The influence of synbiotic (Biomin Imbo) on growth factors and survival rate of Zebrafish (*Danio rerio*) larvae via supplementation with biomar. Global Veterinaria 8(5):503–506

Ouwehand AC, DongLian C, Weijian X, Stewart M, Ni J, Stewart T, Miller LE (2014) Probiotics reduce symptoms of antibiotic use in a hospital setting: a randomized dose response study. Vaccine 32(4):458–463

Paperna I (1991) Diseases caused by parasites in the aquaculture of warm water fish. Annu Rev Fish Dis 1:155–194

Parker RB (1974) Probiotics, the other half of the antibiotic story. Anim Nutr Health 29:4–8

Patel RM, Denning PW (2013) Therapeutic use of prebiotics, probiotics, and postbiotics to prevent necrotizing enterocolitis: what is the current evidence? Clin Perinatol 40(1):11–25

Pérez T, Balcázar JL, Ruiz-Zarzuela I, Halaihel N, Vendrell D, De Blas I, Múzquiz JL (2010) Host–microbiota interactions within the fish intestinal ecosystem. Mucosal Immunol 3(4):355–360

Pérez-Sánchez T, Balcázar JL, Merrifield DL, Carnevali O, Gioacchini G, de Blas I, Ruiz-Zarzuela I (2011) Expression of immune-related genes in rainbow trout (Oncorhynchus mykiss) induced by probiotic bacteria during Lactococcus garvieae infection. Fish Shellfish Immunol 31(2): 196–201

Pérez-Sánchez T, Mora-Sánchez B, Balcázar JL (2018) Biological approaches for disease control in aquaculture: advantages, limitations and challenges. Trends Microbiol 26(11):896–903

Reid G, Sanders ME, Gaskins HR, Gibson GR, Mercenier A, Rastall R, Roberfroid M, Rowland I, Cherbut C, Klaenhammer TR (2003) New scientific paradigms for probiotics and prebiotics. J Clin Gastroenterol 37(2):105–118

Salminen S (1996) Uniqueness of probiotic strains. Newsletter-International Dairy Federation, p 18

Satam SB, Sawant NH, Ghughuskar MM, Sahastrabuddhe VD, Naik VV, Pagarkar AU, Chogale ND, Metar SY, Shinde K, Sadawarte AN, Sawant AN (2018) Ornamental fisheries: a new avenue to supplement farm income. Adv Agric Res Technol J 2:193–197

Schaafsma G (1996) State of the art concerning probiotic strains in milk products. IDF Nutr Newsl 5:23–24

Schrezenmeir J, de Vrese M (2001) Probiotics, prebiotics, and synbiotics—approaching a definition. Am J Clin Nutr 73(2):361–364

Serrano PH (2005) Responsible use of antibiotics in aquaculture. Food & Agriculture Organisation, Geneva

Sharma OP, Bhukar SK (2000) Effect of Aquazyn-TM-1000, a probiotic on the water quality and growth of *Cyprinus carpio* var. *communis (L.)*. Indian J Fish 47(3):209–213

Sharma R, Kapoor B, Sharma P (2019) Biotechnological interventions for sustainable vegetable production under the scenario of climate change. Paper presented at innovative interventions for sustainable vegetable production under changing climate scenario, 3–23 September 2019

Skjermo J, Vadstein O (1999) Techniques for microbial control in the intensive rearing of marine larvae. Aquaculture 177(1-4):333–343

Sperti GS (1971) Probiotics, pub. west point. CT: Avi Co 69:105–107

Steenbergen L, Sellaro R, van Hemert S, Bosch JA, Colzato LS (2015) A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. Brain Behav Immun 48:258–264

Sze CP (2000) Antibiotic use in aquaculture: the Malaysian perspective. Infofish Int 2:24–29

Tuomola E, Crittenden R, Playne M, Isolauri E, Salminen S (2001) Quality assurance criteria for probiotic bacteria. Am J Clin Nutr 73(2):393–398

Uma A, Abraham TJ, Sundararaj V (1999) Effect of a probiotic bacterium, Lactobacillus plantarum on disease resistance of Penaeus indicus larvae. Indian J Fish 46(4):367–373

Van Doan H, Hoseinifar SH, Ringø E, Ángeles Esteban M, Dadar M, Dawood MA, Faggio C (2020) Host-associated probiotics: a key factor in sustainable aquaculture. Rev Fish Sci Aquacult 28(1):16–42

Ventura M, Turroni F, van Sinderen D (2015) Bifidobacteria of the human gut: our special friends. In: Diet-microbe interactions. Academic Press, Cambridge, pp 41–51

Vinderola G, Binetti A, Burns P, Reinheimer J (2011) Cell viability and functionality of probiotic bacteria in dairy products. Front Microbiol 2:70

Wood E (2001) Collection of coral reef fish for aquaria: global trade. In: Conservation issues and management strategies. Marine Conservation Society, Ross-on-Wye, p 80

Xue L, He J, Gao N, Lu X, Li M, Wu X, Liu Z, Jin Y, Liu J, Xu J, Geng Y (2017) Probiotics may delay the progression of nonalcoholic fatty liver disease by restoring the gut microbiota structure and improving intestinal endotoxemia. Sci Rep 7:45176

Zhang M, Kou J, Wu Y, Wang M, Zhou X, Yang Y, Wu Z (2017) Dietary genistein supplementation improves intestinal mucosal barrier function in *Escherichia coli* O78-challenged broilers. J Nutr Biochem 77:108267

Ziaei-Nejad S, Rezaei MH, Takami GA, Lovett DL, Mirvaghefi AR, Shakouri M (2006) The effect of Bacillus spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp Fenneropenaeus indicus. Aquaculture 252(2-4):516–524

Zorriehzahra MJ, Delshad ST, Adel M, Tiwari R, Karthik K, Dhama K, Lazado CC (2016) Probiotics as beneficial microbes in aquaculture: an update on their multiple modes of action: a review. Vet Q 36(4):228–241

7 The Commercial Perspective of Probiotics and Bioremediating Components in Aquaculture Pond Management: A Case Study

Prasenjit Barman, Kalyanbrata Pal, Suman Kumar Halder, and Partha Bandyopadhyay

Abstract

Four bacterial strains, i.e., FB5, FB7, FB9, and FB12, from the GI tract of *Penaeus vannamei* and the strain FB19 were isolated from different *P. vannamei* culture ponds, and their probiotic potentiality and bioremediating capability were studied. Through ribosomal DNA sequence profiling, strains FB5, FB7, FB9, FB12, and FB19 were identified as *Bacillus subtilis*, *Bacillus circulans*, *Bacillus subtilis*, *Bacillus licheniformis*, and *Pseudomonas* sp., respectively. The isolated bacterial strains are able to produce different enzymes like amylase, protease, phytases, and beta-galactosidase that are very essential for shrimp growth and cleanup of the pond bottom condition. All the *Bacillus* strains showed antimicrobial activity against shrimp pathogen like *V. harveyi* MTCC 7954 and *V. vulnificus* MTCC 1145, but *B. subtilis* FB5 highest zones of inhibition against *V. harveyi* MTCC 7954 and *V. vulnificus* MTCC 1145 were 15.3 ± 1.11 mm and 16.1 ± 1.34 mm, respectively. The serum bactericidal activities of nonspecific immunity of shrimps were also measured. Heterotrophic nitrifying and aerobic denitrifying *B. subtilis* FB5 has the ability to remove $NH_4^+ - N$, $NO_3^- - N$, and $NO_2^- - N$ at the rate of 76.27, 77.69, and 64.51%, respectively, in synthetic media, whereas aerobic denitrifying *Pseudomonas* sp. FB19 has the ability to remove 87.14% $NO_2^- - N$ in synthetic medium. The bacterial consortium was applied in *P. vannamei* culture pond, and removal of $NH_4^+ - N$, $NO_3^- - N$, and $NO_2^- - N$ was also recorded at 86.41, 93.94, and 98.30%, respectively. The results attested that all these *Bacillus* strains were potential

P. Barman (✉) · P. Bandyopadhyay
Research and Development, FINRAY BIOTECH INC., Kolkata, West Bengal, India
e-mail: research@finraybiotech.com

K. Pal · S. K. Halder
Department of Microbiology, Vidyasagar University, Midnapore, West Bengal, India

K. K. Behera et al. (eds.), *Prebiotics, Probiotics and Nutraceuticals*,
https://doi.org/10.1007/978-981-16-8990-1_7

probiotics for *P. vannamei* and the removal efficiency of toxic nitrogenous pollutants. It was clearly established that this consortium has bioremediating potentiality for sustainable shrimp culture.

Keywords

Penaeus vannamei · Probiotics · Antagonistic properties · Nonspecific immunity · Nitrogenous pollutant · Bioremediating component

7.1 Introduction

Shrimp aquaculture has quickly developed to a significant industry around the world, not only turning out financial revenue and excellent food item but also giving additional work to many thousands gifted and incompetent laborers. It has been predictable that the world population will arrive at 9 billion by 2050 and hydroponics will have significant part in obliging the expanded interest for food (Godfray et al. 2010). In aquaculture, there have difficulties related with animal illness and the most ideal approach to understand these is regularly through compelling administration rehearses, that is, the board of stock, water, soil, nourishment, and climate (Ringø et al. 2014). Issues identified with infections and crumbling of natural boundaries frequently happen and bring about genuine financial losses. Thus, infections are presently regarded as one of the basic restricting components in shrimp farming. Infections by different members of *Vibrio* genus are the prime factor for shrimp mortality, especially at juvenile stage. It brings about critical low creation of cultivated shrimp and monetary misfortune to business cultivators, which has a financial statement of approximately 25% of the complete world aquaculture item (Kumar et al. 2016). The most dominating microbes in aquaculture are *Vibrio* species like *V. alginolyticus*, *V. harveyi*, *V. parahaemolyticus*, *V. splendidus*, and *V. vulnificus*, which can cause an array of sickness with mortality of up to 100% (Barman et al. 2015). Anti-infection or chemotherapeutic agents are the best option of treatment in the instances of sicknesses brought about by *Vibrio* spp. In any case, constant use of antitoxins or chemotherapeutics in aquaculture has possible adverse impacts especially for the emerging drug resistance of infection causing microbes through horizontal gene transfer (Barman et al. 2015). The significant reason for sickness is because of poor water quality which is due to supplementation of protein rich nutrient in excess for hyper-production of the animal (Boyd 1985; Shimeno et al. 1997). The gathering of ammonium nitrogen is profoundly harmful at any phase of a shrimp culture framework. Unconsumed feed, dead bacterial biomass, and fecal matters are the central point for ammonium nitrogen gathering in shrimp lakes. Furthermore, nitrite is accumulated by chemolithotrophic ammonia oxidizers because of alkali oxidation. For profitability, high stocking density in shrimp cultivation is one of the prime reason for intensification of ammonia level in shrimp culture pond. A raised degree of ammonium nitrogen accumulation affecting growth, molting and Na^+ and K^+-ATPase activities of peneids (Barman et al.

2020). Gill hyperplasia, an antecedent to bacterial disease and in extraordinary cases demise of shrimp, happens at higher harmful degrees of smelling salts in shrimp cultivation frameworks. Unionized alkali is more harmful for shrimp when it goes through the gill film and leads to pH changes in the cytoplasm (Spotte 1979). In shrimp pond, the administration of poisonous metabolites involves concern on the grounds that the everyday explicit discharge of alkali is several folds greater by postlarvae than that of grownups on a weight premise (Barman et al. 2020). As of late, a methodology of utilizing joined probiotics is picking up fame around the world. It is recommended that utilization of advantageous microbes to the pond mud can quicken the deterioration of unwanted natural issues and other side effects through bioremediation and maybe even helps in the increment of oxygen levels (Sonnenholzner and Boyd 2000; Wang and Han 2007). Therefore, improvement of water quality through bioremediation becomes a promising and successful choice in hydroponics (use of microorganisms/chemicals). Commercial microbial strains could upgrade water and residue quality, which eventually improve growth and development of shrimp. In this context, lactic acid producing microbes (*Lactobacillus*, *Carnobacterium*, etc.), *Vibrio*, *Bacillus*, and *Pseudomonas*, were proposed as potential biocontrol agents (Barman et al. 2015). *Bacillus* species were also documented as potential candidate for nitrogen evacuation (Kim et al. 2005; Yang et al. 2011).

In this perspective, the present study was aimed to improve the water quality of *Penaeus vannamei* culture ponds for the enhancement of productivity through the application of enzyme-producing and aerobic nitrifying-denitrifying bacterial consortium in the culture ponds.

7.2 Materials and Methods

7.2.1 Sampling Area

Twenty diverse water and soil residues were procured from various white shrimp (*Penaeus vannamei*) culture ponds situated at the Contai District (21.68°N and 87.55°E), Bay of Bengal Coast, West Bengal, India, and Baliapal (21.65°N and 87.28°E), Subarnarekha waterway locale, Odisha, India, to detach probiotic microbes and heterotrophic nitrifying and high-impact denitrifying microscopic organisms.

7.2.2 Collection of Shrimp Gut Sample for Isolation of Probiotic Bacteria

Sound white shrimps, *Penaeus vannamei* (20 ± 2 gm) (no. 12), were collected from the semi-intensive pond from the aforesaid area and brought into the research facility in live condition for the seclusion of probiotic microorganisms. After arriving at the research facility, hepatopancreas were collected from shrimps and crushed very well with sterile saline water and were taken 1 ml to make serial dilution up to 10^{-8} fold. Then, 100 μl was spread on Brain Heart Infusion Agar media, and plates were incubated at 30 ± 1 °C for 48 h. A total of 12 different morphological colonies were isolated and purified.

7.2.3 Screening of Probiotic Bacteria

Pre-grown colonies of pathogenic strains *V. harveyi* MTCC 7954 and *V. vulnificus* MTCC 1145 were inoculated into Tryptone Soy Broth and incubated for 24 h at 30 °C. Both cultures were diluted in normal saline solution to achieve at the convergence of 10^6 cfu/ml and inoculated in nutrient agar plate through pour plate technique. The young broth culture (of 0.1 ml) of pre-isolated gut bacteria was then separately inoculated as a spot onto the pathogen inoculated plates. After overnight incubation at 30 °C, the clear zones of inhibition of the growth of pathogenic bacteria were measured.

7.2.4 Isolation of Heterotrophic Nitrifying and Aerobic Denitrifying Bacteria

For isolation of nitrifying and denitrifying bacteria, an enrichment culturing process was executed. An inorganic composite culture medium was prepared according to Barman et al. (2017a, b) which was inoculated with 5 ml of soil sediment from shrimp culture pond followed by incubation at 30 °C under shaking (200 revolutions/min) for the enhancement of nitrifying bacterial load. From that, 5 ml spent medium was inoculated into 100 ml of fresh medium and incubated at the said condition for 2 days. Thereafter, the broth culture was diluted and spread on the basal inorganic agar plate. After 2 days of incubation at 30 °C, a total of nine distinct colonies were selected and purely cultured. The organisms are grown on a liquid inorganic medium (at 30 °C), and the efficacy of removal of ammonium nitrogen, nitrate nitrogen, and nitrite nitrogen was periodically measured. To confirm the denitrification efficiency, the isolates were grown on a bromothymol blue (BTB) medium, and the evacuation of $NO_2^- - N$ was measured according to Barman et al. (2017a, b).

7.2.5 Molecular Identification of the Selected Strain

The selected bacterial strains were identified on the basis of 16 rDNA profiling according to Barman et al. (2017a, b).

7.2.6 Enzymatic Assay of Probiotic Bacteria

Alpha-amylase activity was assessed according to Rick and Stegbauer (1974). The supernatant (0.5 ml) was mixed with 0.5 ml of 1.0% soluble starch and incubated at 40 °C for 10 min. After incubation, 1.0 ml of DNS (3,5-dinitrosalicylic acid) reagent was added and heated in boiling water bath (100 °C) for 5 min. The optical density was spectrophotometrically measured at 540 nm. One enzyme unit (U) was defined as the amount of protein that produces 1.0 μM of glucose/min under standard assay condition.

Protease activity was estimated according to Kembhavi and Kulkami (1993) with some modification. To estimate, the same 0.15 ml supernatant was mixed with 0.3 ml of 1% (w/v) casein (in 20 mM Tris-HCl buffer, pH 7.4) and kept at 37 °C for 30 min. The reaction was terminated by adding 0.45 ml of a 10% (w/v) trichloroacetic acid. The absorbance was measured at 280 nm. One unit of protease activity was defined as the amount of enzyme required to liberate 1 μM of tyrosine/min under assay condition.

Phytase activity was measured according to Gulati et al. (2007). In brief, 100 μl of sodium phytate (0.5 w/v in 0.2M sodium acetate buffer, pH 5.5) was mixed with the same volume of supernatant. After 30 min of incubation at 50 °C, the reaction was stopped by adding 200 μl of 15% trichloroacetic acid. The released phosphates were measured quantitatively according to Gulati et al. (2007). One unit of phytase activity was defined as the amount of enzyme needed to liberate 1 μM of phosphate/min under the assay conditions.

Beta-galactosidase activity was estimated by Miller (1972). In brief, 0.5 ml supernatant was mixed with 0.5 ml Na-phosphate buffer (0.05M, pH 6.8) and 1 drop of 0.1% SDS and incubated at 30 °C for 2 min. Thereafter, 0.2 ml ONPG was added. At the point of appearance of yellow color (o-nitrophenol), the reaction was halted by adding 0.5 ml of 1M Na_2CO_3. One unit of beta-galactosidase activity was defined as the amount of enzyme required to liberate 1 μM of ONPG per min under test condition.

7.2.7 Measurement of Heterotrophic Nitrification and Aerobic Denitrification Efficiency

The efficiency of heterotrophic nitrification and aerobic denitrification of the selected bacteria was measured according to Barman et al. (2017a, b).

7.2.8 Confirmation of the Presence of Nitrifying and Denitrifying Gene

The presence of nitrification-related gene hydroxylamine oxidoreductase (*hao*) and denitrification-related gene nitrate reductase (*napA*) and nitrite reductase (*nirS*) in the

Table 7.1 Primer names and sequences used to amplify fragments from *hao*, *napA*, and *nirS* genes in the nitrification pathways

Target gene	Gene names	Primer names	Base of primers (5′–3′)
hao	Hydroxylamine oxidase	haoF1 haoR3	TGCGTGGARTGYCAC AGRTARGAKYSGGCAAA
napA	Periplasmic nitrate reductase	LF716 SR2294	GCNGARATGCACCC GWRTGCCARTGNTC
nirS	Homodimeric cytochrome cd1 nitrite reductase	nirS1F nirS6R	CCTAYTGGCCGCCRCART CGTTGAACTTRCCGGT

selected bacteria was checked by gene-specific amplification according to the method of Barman et al. (2017a, b) (Table 7.1).

7.2.9 Application of Bacterial Consortia in *P. vannamei* Culture Pond

The investigation was performed at the beginning of the month of May 2019 and continued for 30 days at Contai (21.78°N and 87.92°E) in the East Coast of the Bay of Bengal, India, and the pond size was 0.6 ha where the water depth was 1.3 m. Shrimps with average body weight of 10 g and 60 days of culture (DOC) were utilized in the preliminary investigation. Charoen Pokphand (CP) feed was applied to the shrimp pond. The pond was outfitted with a paddle wheel for air circulation. The figured consortium (FB5, FB7, FB9, FB12, and FB19 strains in equivalent focus, 1×10^6 CFU each strain) was added twice (15 days interval) into the treatment ponds.

7.2.10 Collection of Hemolymph and Determination of Serum Antimicrobial Activity

After 30 days of trial, hemolymph was acquired from the ventral piece of the hemocoel according to the method adopted by Rodriguez et al. (1995). The serum sample was collected, and the antimicrobial activity of the serum sample against *Vibrio harveyi* was determined according to Kajita et al. (1990) on Tryptone Soy Agar.

7.2.11 Physicochemical Analysis of Water in *P. vannamei* Culture Pond

Different water parameters like dissolved oxygen, alkalinity, saltiness, pH (water and residue), concentration of ammonia, nitrate, and nitrite of the pond water were estimated by standard techniques (APHA AWWA WPCF 2005) as per the protocol followed by Barman et al. (2015).

7.2.12 Statistical Analysis

Experiments and tests are performed in triplicate, and the data was represented as mean ± standard deviation. The calculation and graph preparation were carried out in MS Excel package 2010.

7.3 Result and Discussion

7.3.1 Isolation and Selection of Probiotic Bacteria from *Penaeus vannamei*

Intestinal bacteria of *Penaeus vannamei* were isolated, and their probiotic potential was investigated by checking their efficacy to inhibit the growth of *Vibrio harveyi* MTCC 7954 and *Vibrio vulnificus* MTCC 1145 (Table 7.2). The results revealed differential inhibitory effect of the investigating bacteria against the two *Vibrio* sp., and the zones of growth inhibition are varied between 2.8 and 9.6 mm. Among them, strains FB5, FB7, FB9, and FB12 imparted zone of growth inhibition of more than 12 mm against the test pathogens and therefore were selected for subsequent study. Literature review revealed that *Bacillus* sp. isolated from the digestive system of shrimp can repress pathogenic strains like *V. harveyi*, *V. vulnificus*, and *V. alginolyticus* (Balcazar and Rojas-Luna 2007; Barman et al. 2011; Sivakumar et al. 2012; Tank et al. 2018). During comparative analysis, it was revealed that the

Table 7.2 Inhibitory effects of the isolates on different pathogenic bacterial strains

Name of the strain	Pathogen	
	Vibrio harveyi MTCC 7954 (zone of inhibition in mm)	*Vibrio vulnificus* MTCC 1145 (zone of inhibition in mm)
Strain FB1	8.4 ± 0.55	8.4 ± 0.37
Strain FB2	7.2 ± 0.32	9.8 ± 0.54
Strain FB3	9.2 ± 0.34	10.1 ± 1.12
Strain FB4	8.9 ± 0.31	11.7 ± 1.42
Strain FB5	15.3 ± 1.11	16.1 ± 1.34
Strain FB6	9.7 ± 0.45	8.7 ± 0.67
Strain FB7	13.2 ± 0.56	12.2 ± 1.17
Strain FB8	10.1 ± 0.31	8.40 ± 0.32
Strain FB9	14.5 ± 1.21	13.3 ± 0.88
Strain FB10	10.4 ± 0.44	9.8 ± 0.43
Strain FB11	10.8 ± 0.32	11.3 ± 1.43
Strain FB12	13.2 ± 0.87	14.7 ± 1.32

strain FB5 showed highest zones of inhibition of 15.3 ± 1.11 mm and 16.1 ± 1.34 mm against *V. harveyi* MTCC 7954 and *V. vulnificus* MTCC 1145, respectively (Table 7.2). This result attested better zone of inhibition as reported by Tank et al. (2018). The reasonable antimicrobial activity of the selected bacterial strain suggested their possible in vivo potential to inhibit the growth of invading pathogenic bacteria in the intestine of *Penaeus vannamei*. Sugita et al. (1996) documented that the aforementioned effects are generally endorsed by any of the following factors, viz., biosynthesized antibiotics, bacteriocin, siderophore, lysozyme, protease, and organic acid singly or in combination.

7.3.2 Isolation of Heterotrophic Nitrifying and Aerobic Denitrifying Bacteria from Water and Soil Sample

Different nine morphological colonies were selected by plating on inorganic medium from the 20 different soil and water samples. The selected nine strains and previously selected four probiotic strains were individually cultured for 24 h on an inorganic medium at 30 °C with shaking condition to study their nitrifying efficiency. After 24 h of the experiment, the ammonium nitrogen removal capability was measured and recorded that the strain FB9 eliminates 44.91 ± 0.51% of ammonium nitrogen which was higher than others (Table 7.3). In this manner, FB9 was chosen for subsequent study. To find out the high-impact denitrifying microbes, the selected nine strains were cultured on Bromothymol Blue (BTB) medium. Among them, four diverse morphological colonies were come into see on BTB plate. Further, these four isolates were cultured at 30 °C aerobically in liquid denitrification medium (DM) and periodically estimated $NO_2^- - N$ removal proficiency to find out the best denitrifying organism. Among them, the strain FB19 exhibited highest $NO_2^- - N$ removal efficiency and hence was selected for subsequent study. Among them, the noteworthy $NO_2^- - N$ removal effective microbes were discovered

Table 7.3 Ammonium nitrogen removal efficiency of the isolates

Strain name	Ammonium nitrogen removal (%)
FB5	0.0
FB7	0.0
FB9	44.91 ± 0.51
FB12	0.0
FB13	23.48 ± 0.62
FB14	28.81 ± 0.25
FB15	35.53 ± 0.45
FB16	29.75 ± 0.63
FB17	19.52 ± 0.49
FB18	36.63 ± 0.39
FB19	28.64 ± 0.37
FB20	31.23 ± 0.55
FB21	29.67 ± 0.68

Table 7.4 Nitrite nitrogen removal efficiency of the isolates

Strain name	Nitrite nitrogen removal (%)
FB18	51.45 ± 0.36
FB19	67.85 ± 0.43
FB20	49.75 ± 0.52
FB21	57.64 ± 0.39

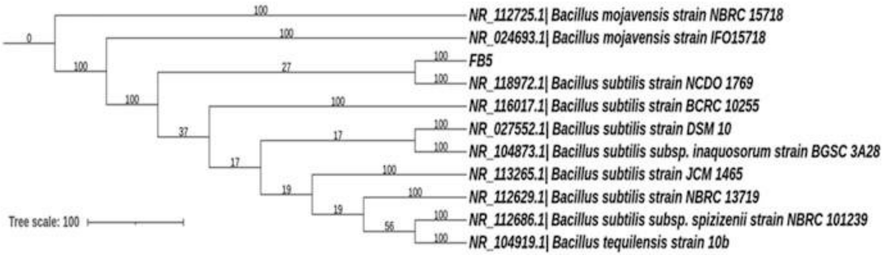


Fig. 7.1 Phylogenetic tree of strain FB5 and related bacteria based on partial 16S rDNA sequence. The tree was constructed using MEGA 4.0

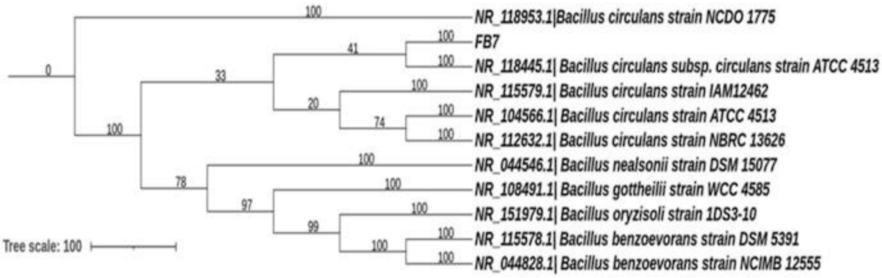


Fig. 7.2 Phylogenetic tree of strain FB7 and related bacteria based on partial 16S rDNA sequence. The tree was constructed using MEGA 4.0

in contrast with different isolates (Table 7.4) and assigned as FB19 just as protected at 4 °C for further analysis.

7.3.3 Identification of the Isolates

For identification of the isolates, the PCR amplicon of partial 16S rDNA was sequenced and subsequently subjected for homology search in NCBI-BLAST. The phylogenetic tree was constructed by a neighbor joining strategy based on 16S rDNA sequence. On the basis of maximum similarity as well as phylogenetic analysis, unknown bacterium FB5 was identified as *Bacillus subtilis* (Fig. 7.1). Likewise, FB7, FB9, FB12, and FB19 were identified as *Bacillus circulans* (Fig. 7.2), *Bacillus subtilis* (Fig. 7.3), *Bacillus licheniformis* (Fig. 7.4), and *Pseudomonas* sp. (Fig. 7.5), respectively. Though both strains FB5 and FB9 were identified

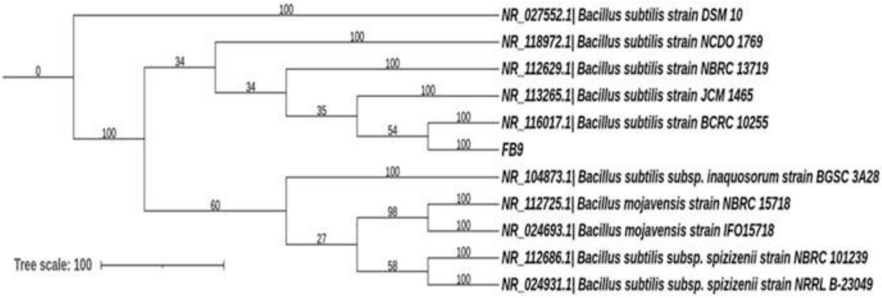


Fig. 7.3 Phylogenetic tree of strain FB9 and related bacteria based on partial 16S rDNA sequence. The tree was constructed using MEGA 4.0

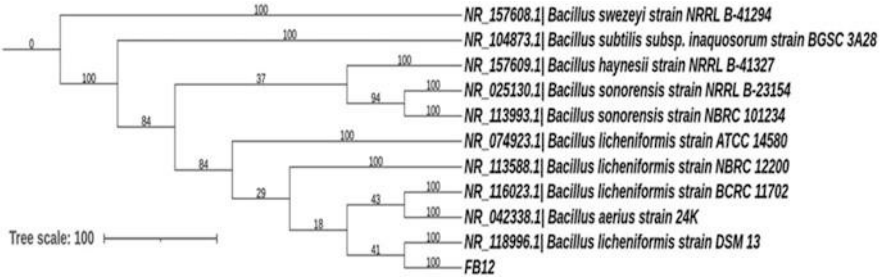


Fig. 7.4 Phylogenetic tree of strain FB12 and related bacteria based on partial 16S rDNA sequence. The tree was constructed using MEGA 4.0

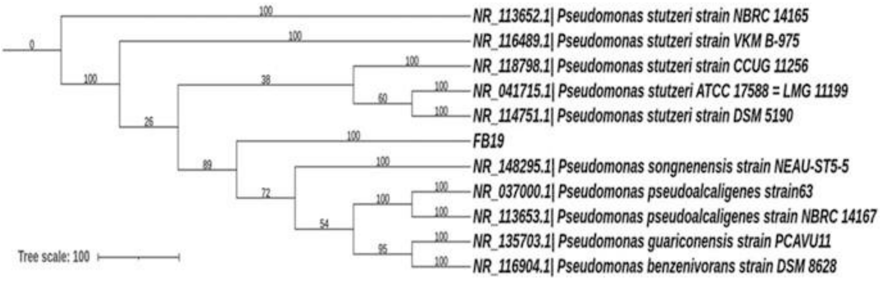


Fig. 7.5 Phylogenetic tree of strain FB19 and related bacteria based on partial 16S rDNA sequence. The tree was constructed using MEGA 4.0

as *Bacillus subtilis*, according to ammonia removal efficiency (Table 7.4), the strain FB9 eliminated 44% within 24 h, while the strain FB5 has no such ability. So it was affirmed that strains FB5 and FB9 both were different strains of *Bacillus subtilis*.

7.3.4 Microbial Enzymes of Probiotics

In animals, after ingestion, processing of food is done by the animal's digestive framework and by microorganisms that reside in the intestinal tract. Literature review attested that microorganisms present in the gastrointestinal tract of fish are powerful makers of proteolytic enzymes. In the current study, probiotic strains biosynthesized an array of digestive enzymes, viz., amylase, protease, phytases, and beta-galactosidase (Table 7.5). Recently, in fish feed, additional (exogenous) enzymes were added for fish health improvement and pulled in broad consideration from feed makers and fish or shrimp consultant. Globally, commercial enzymes are used as an adjunct material to make fish feed (Zheng et al. 2019). Generally macronutrients are the significant property for shrimp health, activities, and growth. Among the macronutrients, 28–57% protein, 23–32% carbohydrate, and 6–7.5% lipids are required for shrimp health, and also it depends on different species of shrimp (Akiyama et al. 1991; Shiau 1998). Leonel and Olmos (2006) reported that different types of enzymes like amylase, protease, cellulase, catalase, lactase, beta-galactosidase, lipase, and phytases can be produced by *Bacillus* sp. Many research works have indicated that production of shrimp was high when enzymes are added in fish feed (Magalhaes et al. 2016; Kemigabo et al. 2018). Generally, the percentages of protein, carbohydrate, and fat in shrimp are 40–50, 20–30, and 5–10, respectively, with other ingredients. The amount of wasted feed is 30–40% in culture pond during the feeding time. The unutilized feed was quickly digested by proteins and maintains the pond to be less toxic. In the shrimp culture pond, polysaccharides and starch degrade by amylase, while phosphorus comes from phytate by the help of phytases which act as a source of mineral and phosphorus containing amino acid. Plant protein and animal protein are digested by protease enzyme in the shrimp culture pond. Beta-galactosidase helps in bioconversion of disaccharide lactose into glucose and galactose which were subsequently metabolized (Barman et al. 2015; Zheng et al. 2019).

Table 7.5 Study of enzymatic profile of the isolates

Microbial enzymes	Isolated probiotics *B. subtilis* FB5	*B. circulans* FB7	*B. subtilis* FB9	*B. licheniformis* FB12
Amylase	+	+	+	–
Protease	+	–	+	+
Phytase	+	+	+	+
Beta-galactosidase	+	+	+	–

7.3.5 Nitrification and Denitrification Potentiality of *B. subtilis* FB9 and *Pseudomonas* sp. FB19

The ammonium nitrogen evacuation limit of FB9 was surveyed at 30 °C for 24 h on an inorganic medium under high-impact circumstance. The strain *B. subtilis* FB9 removed 76.27% of NH_4^+-N in 39 h, and 2.3 mg/l/h was the nitrifying rate (Fig. 7.6a). At the same time, the concentration of $NO_3^- - N$ was measured and found that 77.69% were reduced where $NO_2^- - N$ was decreased to 64.51% by *B. subtilis* FB9 (Fig. 7.6b). It was accounted for that diverse bacterial genera can eliminate $NH_4^+ - N$, for example, *K. pneumonia* CF-S9, *Pseudomonas alcaligenes* AS-1, and *Rhodococcus* sp. CPZ24 (Su et al. 2006; Chen et al. 2012; Padhi et al. 2013). Literature review endorsed that nitrification efficiency of *B. subtilis* A1 (0.3 mg/l/h), *B. cereus* PB45 (1.8 mg/l/h), and *Bacillus* sp. LY (0.43 mg/l/h) were comparatively less than the prsent study (Zhao et al. 2010; Yang et al. 2011; Barman et al. 2016). Yet, *B. subtilis* FB9 exhibited foremost $NH_4^+ - N$ evacuation proficiency (2.3 mg/l/h) among different *Bacillus* investigated in this study. The nitrification efficiency increased gradually in a time-dependent manner and reached the peak at 15 h and steadily decreased thereafter. At the first 12 h, the amount of $NO_2^- - N$ increased because of nitrate being augmented at the same time. After that, the bacterial cell density increased, and $NO_3^- - N$ and $NO_2^- - N$ concentration reduced. Heterotrophic nitrification and aerobic denitrification processes regulate the $NO_3^- - N$ and $NO_2^- - N$ concentration in a balanced condition. But in shrimp culture pond, $NO_2^- - N$ is a very toxic compound and very stressful to shrimp growth. For this reason, *Pseudomonas* sp. FB19 was also used to accelerate the denitrification process. The denitrification capacity of FB19 was evaluated in denitrification medium at 30 °C for 35 h under aerobic condition. *Pseudomonas* sp. FB19 removed 87.14% of $NO_2^- - N$ in 35 h with 0.34 mg/l/h denitrification rate (Fig. 7.6c), which is higher than *B. cereus* PB88 reported by Barman et al. (2017a, b). Prior idea was that denitrification cycle happens carefully under the anaerobic condition (Li et al. 2005). Sufficient amount of dissolved oxygen (DO) is very important in intensive shrimp pond because aquatic animals need a certain DO concentration for their survival (Li et al. 2008). So supplementation of efficient denitrifying microbes is an excellent strategy to lessen the $NO_2^- - N$ in shrimp culture pond.

7.3.6 Nitrifying and Denitrifying Gene Amplification

To complete the nitrifying and denitrifying process, three genes, viz., hydroxylamine oxidoreductase (*hao*), periplasmic nitrate reductase (*napA*), and nitrite reductase (*nirS*), are responsible. After PCR amplification, three distinct brands around 1380 bp, 700 bp, and 560 bp were visualized through electrophoresis which correspond to *nirS*, *napA*, and *hao* genes, respectively (Fig. 7.7). The expression of these genes confirmed that *B. subtilis* FB9 is able to complete the nitrification and denitrification

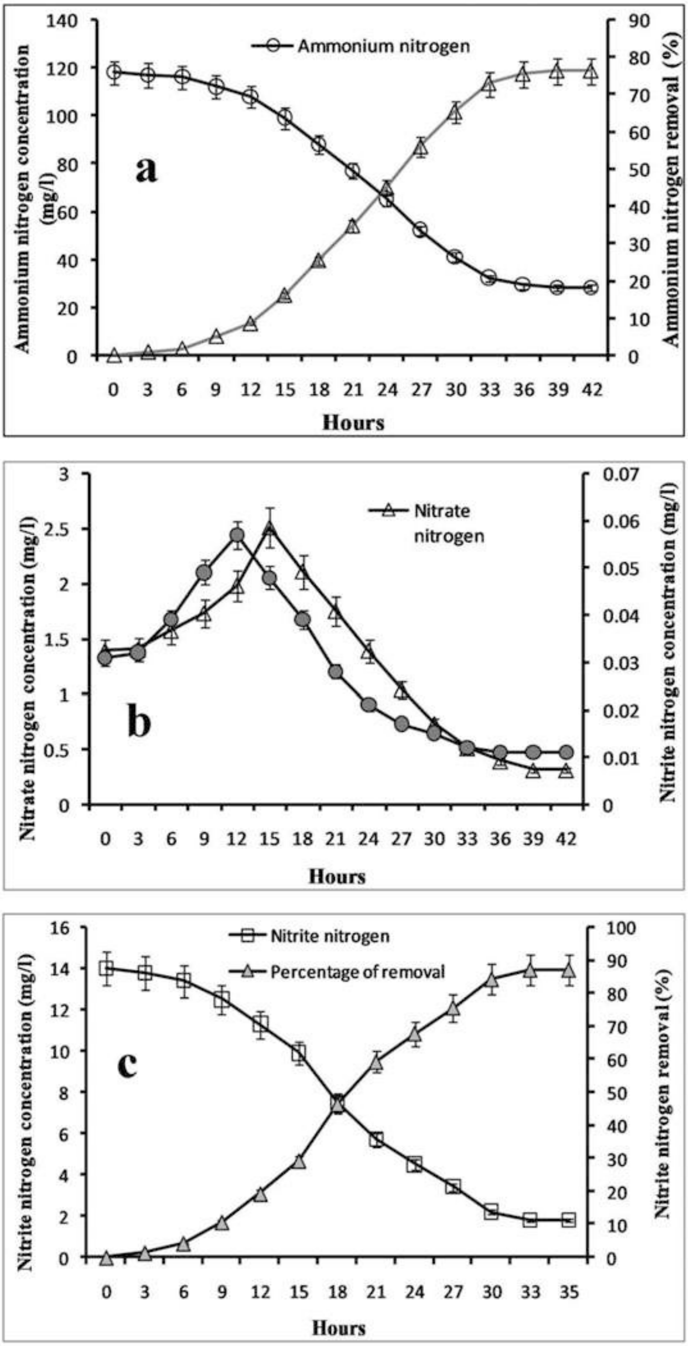


Fig. 7.6 Nitrifying potentiality of *B. subtilis* FB9 in basal inorganic medium. (**a**) Ammonium nitrogen degradation rate and percentage of ammonium nitrogen removal; (**b**) nitrate nitrogen degradation and nitrite nitrogen degradation rate; and (**c**) denitrifying efficiency of *Pseudomonas* sp. FB19

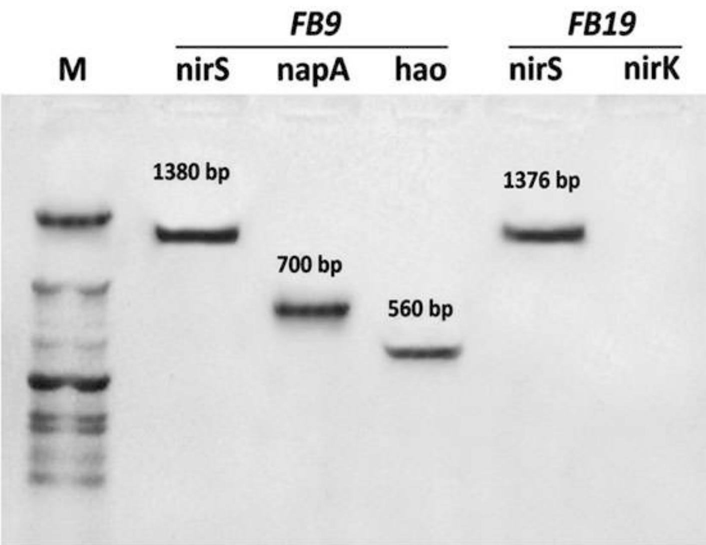


Fig. 7.7 PCR amplification of nitrite reductase (*nirS*), periplasmic nitrate reductase (*napA*), and hydroxylamine oxidase (*hao*) genes in *B. subtilis* FB9 and *nirS* and *nirK* of *Pseudomonas* sp. FB19 (M 100-bp ladder)

process. On the other hand, *nirS* gene of *Pseudomonas* sp. FB19 was also searched for the confirmation of aerobic denitrification process. The amplification of *nirS* gene confirms that it is present in FB19 (Fig. 7.7). A comparative kind of findings was reported on *K. pneumonia* CF-S9 (Padhi et al. 2013) and *B. cereus* PB45 (Barman et al. 2016). From the experiment, it was confirmed that the abovementioned genes were expressed in the selected bacteria.

7.3.7 Serum Bactericidal Activity

After a 30-day trial, the impact of potential probiotic four strains like *B. subtilis* FB5, *B. circulans* FB7, *B. subtilis* FB9, and *B. licheniformis* FB12 to trigger nonspecific defense of shrimp was evaluated by studying the bactericidal efficacy of serum. The result revealed that bactericidal efficacy of the serum of probiotic-treated shrimp (21 ± 0.35 mm) was significantly higher than the efficacy of serum collected from shrimp of control pond (4 ± 0.21 mm). The outcomes acquired in this examination not just help in the utilization of probiotics FB5, FB7, FB9, and FB12 for better zone of hindrance yet additionally affirmed to be a significant immunostimulant in *P. vannamei.* Our result was in accordance with the report of Robertsen et al. (1994). Wang et al. (1999) and Barman et al. (2011) independently theorized that *Bacillus* sp. is potential probiotic organisms which have immense capabilities to upgrade water quality of the shrimp culturing pond and has antimicrobial activity. Our study is in accordance with the aforementioned reports. In this manner, the bacilli are thought to estrange possible microorganisms in the oceanic conditions. It was documented in many cases that cultured microbes are unable to survive in the native environment due to antagonistic effect of other indigenous microbes (Barman et al. 2011; Austin and Al-Zahnari 1988). In this context, the experimental bacteria have the capacity to survive in the natural environment which endorses their real world application. The bacilli enhanced the growth of cultivated species by less utilization of chemicals into the pond (Wang et al. 1999).

7.3.8 Study of Water Quality Parameters of Pond

Throughout the trial periods, different parameters related to water quality were tested periodically for both treatment and control ponds along with examination of shrimp health status. From the observation, it was found that the pH of consortium treated pond varied from 8.0 to 8.3 and this range is very helpful for *P. vannamei* cultivation, but in untreated pond at 90 days, pH reached 8.7 (Table 7.6). Previous work suggested that 6.8–8.7 pH was perfect for shrimp growth and health (Ramanathan et al. 2005) where 7.5–8.5 pH range was the finest for shrimp cultivation (Reddy 2000). Present study also proved that consortium treated pond carried the perfect pH ranges. Another important parameter for intensive shrimp culture is DO, and 4–6 mg/l level is desirable for shrimp cultivation (Boyd and Pillai 1984). During the investigation, more than 5 mg/l DO was kept throughout the trial in both treated and control ponds due to paddle wheel air circulation. Mixture of bicarbonate, carbonate, and hydroxide is called alkalinity in the water. Boyd and Pillai (1984) reported that alkalinity plays a vital role in the water body. Alkalinity of the treatment pond varied from 185 to 195 ppm when bacterial consortium was used (Table 7.6). Hardness is an another important parameter for shrimp culture, and it was found that in treatment pond it varied from 720 to 750 ppm, while in control pond hardness reached to 840 ppm (Table 7.6). In the treated pond, the total dissolved solids were estimated to be 25 ppm after 90 days of culture which is considered to be safe according to Tharavathy (2014). In contrast, the same was estimated to be 55 ppm at the same time in the control pond which is beyond the threshold level (Table 7.6). For the cultivation of shrimp, development of phytoplankton through the application of mineral conditioner along with unutilized feed, fecal matter, and manures collectively maximizes the quantity of solid matter in the pond. During this experiment, salinities decreased from 18 to 16 ppt in both ponds. Shrimp body weight increased in treated pond than control pond because of bacterial consortium which helps to maintain good water quality, reduced ecological pressure, and shedding of shrimp (Table 7.6). In the control pond, ammonium nitrogen removal efficiency was maximum 86.41%, whereas in control pond the removal efficiency was only 27.5% because consortium contains nitrifying bacteria (Table 7.6). At the same time, nitrate nitrogen was decreased from 0.52 ± 0.002 ppm to 0.011 ± 0.004 ppm, whereas in control pond nitrate nitrogen reached from 0.51 ± 0.003 ppm to 0.93 ± 0.003 ppm at end of the trial (Table 7.6). Simultaneously, nitrite nitrogen removal efficiency of 98.30% and 63.88% was recorded in both treated and control ponds,

Table 7.6 Study of water quality parameter and nitrogenous waste removal efficiency by the bacterial consortium

Water parameter	Experimental pond	Before application	After application								
			First dose				Second dose				
		Day 60	Day 61	Day 65	Day 69	Day 73	Day 75	Day 79	Day 83	Day 87	Day 90
pH	Treatment	8.5 ± 0.03	8.1 ± 0.02	8.1 ± 0.03	8.2 ± 0.02	8.3 ± 0.03	8.1 ± 0.03	8.1 ± 0.02	823 ± 0.04	8.2 ± 0.04	8.3 ± 0.02
	Control	8.5 ± 0.04	8.4 ± 0.03	8.3 ± 0.04	8.5 ± 0.02	8.5 ± 0.02	8.5 ± 0.03	8.6 ± 0.02	8.5 ± 0.04	8.6 ± 0.04	8.6 ± 0.03
DO (mg/l)	Treatment	5.5 ± 0.04	5.5 ± 0.04	5.6 ± 0.04	5.5 ± 0.04	5.6 ± 0.02	5.6 ± 0.04	5.5 ± 0.04	5.5 ± 0.03	5.6 ± 0.03	5.5 ± 0.04
	Control	5.4 ± 0.03	5.5 ± 0.03	5.6 ± 0.02	5.4 ± 0.04	5.3 ± 0.04	5.3 ± 0.02	5.3 ± 0.04	5.3 ± 0.02	5.4 ± 0.04	5.3 ± 0.04
Alkalinity (ppm)	Treatment	196 ± 1.3	194 ± 1.2	188 ± 1.2	185 ± 1.3	189 ± 1.4	186 ± 1.4	185 ± 1.3	188 ± 1.3	192 ± 1.3	195 ± 1.3
	Control	195 ± 1.4	196 ± 1.3	196 ± 1.2	197 ± 1.3	197 ± 1.2	198 ± 1.4	198 ± 1.2	202 ± 1.3	205 ± 1.3	207 ± 1.1
Hardness (mg/l)	Treatment	730 ± 2.1	729 ± 1.4	725 ± 1.4	720 ± 1.3	726 ± 1.3	729 ± 1.3	726 ± 1.4	723 ± 1.4	727 ± 1.4	729 ± 1.3
	Control	732 ± 2.2	732 ± 1.3	734 ± 1.4	736 ± 1.4	741 ± 1.3	743 ± 1.2	744 ± 1.3	747 ± 1.3	749 ± 1.2	750 ± 1.1
TDS (ppm)	Treatment	30 ± 0.4	29 ± 0.3	26 ± 0.3	25 ± 0.4	26 ± 0.4	28 ± 0.3	26 ± 0.3	25 ± 0.4	26 ± 0.4	29 ± 0.3
	Control	30 ± 0.4	30 ± 0.2	32 ± 0.2	34 ± 0.2	36 ± 0.3	39 ± 0.2	43 ± 0.3	45 ± 0.4	47 ± 0.3	50 ± 0.2
Salinity (ppt)	Treatment	18 ± 0.11	18 ± 0.11	18 ± 0.15	18 ± 0.14	17 ± 0.18	617 ± 0.17	617 ± 0.14	17 ± 0.16	17 ± 0.16	16 ± 0.14
	Control	18 ± 0.12	18 ± 0.13	18 ± 0.15	18 ± 0.14	17 ± 0.13	617 ± 0.17	617 ± 0.15	17 ± 0.12	17 ± 0.16	16 ± 0.16

ABW (gm)	Treatment	11 ± 0.07	11 ± 0.06	12 ± 0.07	13 ± 0.05	15 ± 0.07	16 ± 0.08	18 ± 0.04	19 ± 0.06	20 ± 0.04	21 ± 0.06
	Control	12 ± 0.04	12 ± 0.06	12 ± 0.04	13 ± 0.03	13 ± 0.05	14 ± 0.06	14 ± 0.04	15 ± 0.03	16 ± 0.06	17 ± 0.03
Ammonium nitrogen (ppm)	Treatment	0.81 ± 0.004	0.29 ± 0.003	0.15 ± 0.004	0.25 ± 0.005	0.42 ± 0.004	0.63 ± 0.006	0.28 ± 0.004	0.11 ± 0.005	0.22 ± 0.003	0.31 ± 0.004
	Control	0.80 ± 0.005	0.80 ± 0.004	0.82 ± 0.006	0.84 ± 0.005	0.85 ± 0.004	0.89 ± 0.004	0.90 ± 0.003	0.94 ± 0.004	0.99 ± 0.005	1.02 ± 0.005
Nitrate nitrogen (ppm)	Treatment	0.52 ± 0.002	0.044 ± 0.003	0.012 ± 0.004	0.027 ± 0.002	0.041 ± 0.004	0.069 ± 0.003	0.011 ± 0.004	0.025 ± 0.002	0.031 ± 0.003	0.042 ± 0.002
	Control	0.51 ± 0.003	0.52 ± 0.004	0.53 ± 0.003	0.58 ± 0.004	0.61 ± 0.003	0.66 ± 0.003	0.73 ± 0.003	0.81 ± 0.004	0.88 ± 0.005	0.93 ± 0.003
Nitrite nitrogen (ppm)	Treatment	0.71 ± 0.002	0.21 ± 0.004	0.014 ± 0.003	0.021 ± 0.003	0.036 ± 0.004	0.058 ± 0.003	0.012 ± 0.003	0.021 ± 0.002	0.033 ± 0.003	0.043 ± 0.004
	Control	0.72 ± 0.003	0.72 ± 0.003	0.78 ± 0.004	0.84 ± 0.003	0.89 ± 0.003	0.92 ± 0.005	0.96 ± 0.003	1.03 ± 0.004	1.13 ± 0.004	1.18 ± 0.005

respectively (Table 7.6). The concentrations of ammonium nitrogen and nitrite nitrogen which are safe for shrimp culture are <1 and 0.25 mg/l, respectively (Chien 1992), and excess amount of these two nitrogenous toxic compounds is the most stressful situation for shrimp culture. The experimental result revealed that concentration of ammonium nitrogen and nitrite nitrogen has been maintained under control as the consortium bacteria performed together in the treated pond.

7.4 Conclusion

From the current investigation, it can be concluded that the bacterial consortium has an essential function in the development, endurance, and illness opposition of the white shrimp, *P. vannamei*, by keeping up better water quality in the culturing pond. It was apparent that the consortium can hinder the development of fish microorganisms and lessen the nitrogenous toxins in the water pool. It tends to be inferred that the consortium might be a decent commercial probiotic specialist as a biocontrol agent and create a sustainable physicochemical environment for better growth of white shrimp.

References

Akiyama DM, Dominy WG, Lawrence AL (1991) Penaeid shrimp nutrition for the commercial feed industry: revised. In: Akiyama DM, Tan RKH (eds) Proceedings of the aquaculture feed processing and nutrition workshop. American Soybean Association, Singapore, pp 80–98

APHA AWWA WPCF (2005) Standard methods for the examination of water and wastewater, 21st edn. American Public Health Association, Washington D.C.

Austin B, Al-Zahnari AMJ (1988) The effect of antimicrobial compounds of the gastrointestinal microflora of rainbow trout, Salmo gairdneri Richardson. J Fish Biol 33:1–14

Balcazar JL, Rojas-Luna T (2007) Inhibitory activity of probiotic *Bacillus subtilis* UTM 126 against *Vibrio* species confers protection against Vibriosis in juvenile shrimp (*Litopenaeus vannamei*). Curr Microbiol 55(5):409–412

Barman P, Banerjee A, Bandyopadhyay P, Mondal KC, Das Mohapatra PK (2011) Isolation, identification and molecular characterization of potential probiotic bacterium, *Bacillus subtilis* PPP 13 from *Penaeus monodon*. Biotechnol Bioinformatics Bioeng 1:473–482

Barman P, Bandyopadhyay P, Mondal KC, Das Mohapatra PK (2015) Water quality improvement of *Penaeus monodon* culture pond for higher productivity through bioremediation. Acta Biol Szeged 59(2):169–177

Barman P, Kati A, Mandal AK, Bandyopadhyay P, Das Mohapatra PK (2016) Biopotentiality of *Bacillus cereus* PB45 for nitrogenous waste detoxification in *ex situ* model. Aquac Int. https://doi.org/10.1007/s10499-016-0105-y

Barman P, Bandyopadhyay P, Kati A, Paul T, Mandal AK, Mondal KC, Das Mohapatra PK (2017a) Characterization and strain improvement of aerobic denitrifying EPS producing bacterium *Bacillus cereus* PB88 for shrimp water quality management. Waste Biomass Valori 9(8): 1319–1330. https://doi.org/10.1007/s12649-017-9912-2

Barman P, Raut S, Sen SK, Shaikh U, Bandyopadhyay P, Das Mohapatra PK (2017b) Effect of a three-component bacterial consortium in white shrimp farming for growth, survival and water quality management. Acta Biol Szeged 61(1):35–44

Barman P, Das Mohapatra PK, Bandyopadhyay P (2020) Application of nitrifying and denitrifying bacterial consortium for nitrogenous waste removal from shrimp culture pond for sustainable cultivation. J Hazard Toxic Radioact Waste 24(4):04020041

Boyd CE (1985) Chemical budgets for channel catfish ponds. Trans Am Fish Soc 114:291–298

Boyd CE, Pillai VK (1984) Water quality management in aquaculture. Special Publ. No. 22, 16. Central Marine Research Institute, Cochin

Chen P, Li J, Li QX, Wang Y, Li S, Ren T, Wang L (2012) Simultaneous heterotrophic nitrification and aerobic denitrification by bacterium *Rhodococcus* sp. CPZ24. Bioresour Technol 116:266–270

Chien YH (1992) Water quality requirements and management for marine shrimp culture. technical bulletin: marine shrimp pond management: a review. U.S. Wheat Associates, American Soybean Association, Singapore, pp 83–122

Godfray HC, Beddington JJR, Crute IR, Haddad L, Lawrence DJ, Muir F, Pretty J, Robinson S, Thomas SM, Toulmin C (2010) Food security: the challenge of feeding 9 billion people. Science 327(5967):812–818

Gulati HK, Chadha BS, Saini HS (2007) Production and characterization of thermostable lkaline phytase from *Bacillus laevolacticus* isolated from rhizospheric soil. J Ind Microbiol Biotechnol 34:91–98

Kajita Y, Sakao M, Atsuta S, Kobayashi M (1990) The immunomodulatory effects of levamisole on Rainbow Trout, *Oncorhynchus mykiss*. Fish Pathol 25(2):93–98

Kembhavi AA, Kulkami A (1993) Salt tolerant and thermostable alkaline protease from *Bacillus subtilis* NCIM No. 64. Appl Biochem Biotechnol 38:83–92

Kemigabo C, Abdeltawwab M, Lazaro JW, Sikawa D, Masembe C, Kang'Ombe J (2018) Combined effect of dietary protein and phytases levels on growth performance, feed utilization, and nutrients digestibility of African catfish, *Clarias gariepinus* (B.), reared in earthen ponds. J Appl Aquac 30(3):211–226

Kim JK, Paik KJ, Cho KS, Nam SW, Park TJ (2005) Aerobic nitrification-denitrification by heterotrophic *Bacillus* strains. Bioresour Technol 96:1897–1906

Kumar V, Roy S, Meena DK, Sarkar UK (2016) Application of probiotics in shrimp aquaculture: importance, mechanisms of action, and methods of administration. Rev Fish Sci Aquac 24(4): 342–368. https://doi.org/10.1080/23308249.2016.1193841

Leonel OS, Olmos SJ (2006) The functional property of *Bacillus* for shrimp feeds. Food Microbiol 23:519–525

Li P, Zhang S, Liu DL (2005) Study progress of bacterial aerobic denitrification. J Microbiol 25:60–64

Li YQ, Zhang HY, Li J, Wang QY, Li ZD (2008) Utilization of liquid oxygen in intensive shrimp aquaculture. Fish Sci 27:401–403

Magalhaes R, Lopes T, Martins N, Dıaz-Rosales P, Couto A, Pousao-Ferreira P, Oliva-Teles A, Peres H (2016) Carbohydrases supplementation increased nutrient utilization in white seabream (*Diplodus sargus*) juveniles fed high soybean meal diets. Aquaculture 463:46–50

Miller JH (1972) Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor

Padhi SK, Tripathy S, Sen R, Sinha Mahapatra A, Mohanty S, Maiti NK (2013) Characterisation of heterotrophic nitrifying and aerobic denitrifying Klebsiella pneumonia CF-S9 strain for bioremediation of wastewater. Int Biodeterior Biodegrad 78:67–73

Ramanathan N, Padmavathy P, Francis T, Athithian S, Selvaranjithan N (2005) Manual on polyculture of tiger shrimp and in freshwater. Fisheries College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Chennai

Reddy R (2000) Culture of the tiger shrimp *Penaeus monodon* (Fabricius) in low saline waters. Master's thesis, Dept. of Marine Biology, Annamalai Univ

Rick W, Stegbauer HP (1974) Methods of enzymatic analysis, 2nd edn. Academic, New York, p 885

Ringø E, Rolf EO, Jensen I, Romero J, Lauzon HL (2014) Application of vaccines and dietary supplements in aquaculture: possibilities and challenges. Rev Fish Biol Fish 24(4):1005–1032

Robertsen B, Engstad R, Jørgensen JB (1994) ß-glucans as immunostimulants in fish. In: Stolen JS, Fletcher TC (eds) Modulators of fish immune responses, Models for environmental toxicology, biomarkers, immunostimulators, vol 1. SOS Publications, Fair Gaven, pp 83–99

Rodriguez J, Boulo V, Mialhe E, Bachere E (1995) Characterisation of shrimp haemocytes and plasma components by monoclonal antibodies. J Cell Sci 108:1043–1050

Shiau SY (1998) Nutrient requirements of penaeid shrimps. Aquaculture 164:77–93

Shimeno S, Shikata T, Hosokawa H, Masumoto T, Kheyyali D (1997) Metabolic response to feeding rates in common carp, *Cyprinus carpio*. Aquaculture 151:371–377

Sivakumar N, Sundararaman M, Selvakumar G (2012) Probiotic effect of *Lactobacillus acidophilus* against Vibriosis in juvenile shrimp (*Penaeus monodon*). Afr J Biotechnol 11(91):15811–15818

Sonnenholzner S, Boyd CE (2000) Managing the accumulation of organic matter deposited on the bottom of shrimp ponds. Do chemical and biological probiotics really work? World Aquac 31: 24–28

Spotte S (1979) Seawater aquariums: the captive environment. John Wiley & Sons, New York

Su JJ, Yeh KS, Tseng PW (2006) A strain of Pseudomonas sp. isolated from piggery wastewater treatment systems with heterotrophic nitrification capability in Taiwan. Curr Microbiol 53:77–81

Sugita H, Matsuo N, Shibuya K, Deguchi Y (1996) Production of antibacterial substances by intestinal bacteria isolated from coastal crab and fish species. J Mar Biotechnol 4:220–223

Tank PR, Vadher KH, Patel MP (2018) Isolation of probiotic bacteria from gastrointestinal tract of pacific white shrimp *Litopenaeus vannamei* and antibacterial activity of probiotic bacteria against *Vibrio* spp. J Entomol Zool 6(4):974–978

Tharavathy MC (2014) Water quality management. Acta Biol Indica 3(1):536–540

Wang YB, Han JZ (2007) The role of probiotic cell wall hydrophobicity in bioremediation of aquaculture. Aquaculture 269:349–354

Wang Q, White BL, Redman RM, Lightner DV (1999) Per os challenge of *Litopenaeus vannamei* postlarvae and *Farfantepenaeus duorarum* juveniles with six geographic isolates of white spot syndrome virus. Aquaculture 170:179–194

Yang XP, Wang SM, De-W Z, Zhou LX (2011) Isolation and nitrogen removal characteristics of an aerobic heterotrophic nitrifying–denitrifying bacterium, *Bacillus subtilis* A1. Bioresour Technol 102:854–862

Zhao B, He YL, Hughes J, Zhang XF (2010) Heterotrophic nitrogen removal by a newly isolated *Acinetobacter calcoaceticus* HNR. Bioresour Technol 101:5194–5200

Zheng CC, Wu JW, Jin ZH, Ye ZF, Yang S, Sun YQ, Fei H (2019) Exogenous enzymes as functional additives in finfish aquaculture. Aquac Nutr. https://doi.org/10.1111/anu.12995

Prebiotics as Promising Therapeutics for Treating Gut-Related Disorders: Biochemical and Molecular Perspectives

8

Ritwik Patra, Saubhik Mitra, Nabarun Chandra Das, and Suprabhat Mukherjee

Abstract

Prebiotic is classically defined as a nondigestible food element that selectively promotes the growth and functionality of the colon microflora and eventually improves host health. It mainly consists of carbohydrates like oligosaccharides, various peptides, and also dietary fibers. Prebiotics have been widely used as nutraceuticals having essential roles in the enhancement of gut microbiota and also work as therapeutics against various inflammatory and infectious diseases associated with the gut. The objective of this chapter is to focus on the biochemical characteristics of various types of prebiotics that are very much essential in our daily diet and their use as nutraceuticals to promote the growth of beneficial microbiota to protect the gut epithelial and mucosal layer against the damage from invading pathogens and toxins. It has been found that the use of various prebiotics as a supplementary diet in both human and animal models significantly regulates the growth of *Bifidobacterium* and *Lactobacillus* and increases the fermentation within the gut leading to the generation of SCFAs and immunomodulation of the host immune system. It is used as a therapeutic agent for the inflammatory bowel disease (IBD) and also inhibits the progression of cancer. Furthermore, it also increases the efficiency of digestion and absorption of nutrients and also protects against pathogenic infections.

Ritwik Patra and Saubhik Mitra contributed equally with all other contributors.

R. Patra · S. Mitra · N. C. Das · S. Mukherjee (✉)
Integrative Biochemistry and Immunology Laboratory, Department of Animal Science, Kazi Nazrul University, Asansol, West Bengal, India
e-mail: suprabhat.mukherjee@knu.ac.in

K. K. Behera et al. (eds.), *Prebiotics, Probiotics and Nutraceuticals*,
https://doi.org/10.1007/978-981-16-8990-1_8

Keywords

Prebiotics · Nutraceuticals · Inflammatory bowel disease (IBD) · Colon cancer · Infectious disease · Therapeutics

8.1 Introduction

Prebiotics were first defined by Gibson and Roberfroid (1995) as “undigestible food ingredients which can provide benefits to the host which can also motivate the development of discriminating probiotics in the colon and promote the health of the host” (Gibson and Roberfroid 1995). The last part of the gut, called the large intestine, is mainly concerned with the absorption of water along with any left behind nutrients, storing undigested food and eventually discarding them out of the body (Manning and Gibson 2004). The large bowel is also responsible for the absorption of several vitamins and minerals and also the recycling of some portion of undigested matters. This structure also houses a diverse array of microflora within, consisting of more than 1000 species of microorganisms that include bacteria, fungi, protozoa, etc. performing various functions. Slow movement of a substantial quantity of undigested food and almost neutral pH helps the microbial inhabitants to flourish (Cummings and Macfarlane 1991; Slavin 2013). Gut microflora are responsible for the fermentation of indigestible long-chain compounds, especially carbohydrates, to shorter-chain fatty acids, causing a passive absorption of the end products. The fermentation causes the production of bowel gases (Gibson 1998). They are also responsible for the synthesis of several vitamins in substantial quantities (Gibson and Roberfroid 1995). A variety of substrates that include undigested starch and non-starch dietary fibers, other polysaccharides or oligosaccharides, proteins, etc. are fermented by the gut microflora. The proteolytic and saccharolytic fermentation by the microorganisms results in the production of phenolic end products or short-chain fatty acids like acetates or butyrates, respectively (Manning and Gibson 2004). Now these nondigestible food constituents providing substrates to the gut microflora are collectively known as “prebiotics.” A prebiotic is classically defined as a nondigestible food element that selectively promotes the growth and functionality of the colon microflora and eventually improves host health (Manning and Gibson 2004; Roberfroid 2007).

A good prebiotic needs to fulfill the following criteria:

- It must be resistant to gastric enzymes and acidity and must not be easily absorbed while passing through the GI tract. Resistance here need not necessarily imply completely indigestible food ingredients, but a substantial quantity of matter must be left in the large intestine for the microbes to ferment.
- Must be available for fermentation by gut microbiota.

- Must selectively stimulate the growth and activity of the beneficial microorganisms.
- Prebiotics, therefore, stimulate the growth of indigenous gut microflora, rather than the introduction of any external organism.

8.2 Biochemical Characteristics of Various Types of Prebiotics

Prebiotics are of many types, mostly being carbohydrates; others include peptides and ester or ether forms of lipids. Their chemical nature prevents absorption at the upper GI tract (Roberfroid 2007). Resistant starch, non-starch polysaccharides or fibers, and oligosaccharides (inulin or FOS, GOS, etc.) occupy the major chunk among prebiotics (Davani-Davari et al. 2019).

8.2.1 Resistant Starch

Dietary carbohydrates can be broadly classified according to their availability to digestion into (a) available carbohydrates (digested by gastric enzymes and are absorbed in the small intestine) or (b) resistant starch (leaves small intestine undigested and almost unabsorbed and reaches the colon) (Englyst et al. 2007; Zhang and Hamaker 2009; Lee et al. 2013). Resistant starch has a similar attribute as of soluble and fermentable fiber. In many cases, starch retrogradation forms partially gelatinous linear molecule of α-1,4-D-glucan that contributes hugely to the resistant starch portion of the diet (Fuentes-Zaragoza et al. 2011; Wang et al. 2015).

Resistant starch is classified into four classes, designated as RS 1–4 (Zaman and Sarbini 2016), though some authors added a fifth class named RS 5 to the cluster (Fuentes-Zaragoza et al. 2011):

RS 1: These are resistant starches that are locked within cell walls of the food making them almost unavailable to digestion.
RS 2: Different raw uncooked or undercooked food contains these starch granules that are not easily digestible due to their structural or conformational properties.
RS 3: These are thermally stable starch granules often generated after cooling of cooked food, so they are retrograded or gelatinized.
RS 4: A group of chemically derived artificial starches which are results of re-polymerization, esterification, etherization, etc., leading to very low digestibility.
RS 5: These are conjugates of starch and lipids, not susceptible to alpha-amylase digestion (Fuentes-Zaragoza et al. 2011).

The presence of high amylose content, due to its compact linear structure and hydrogen bond linkages, confers to resistant starch's increased resistance to enzyme digestion (Zaman and Sarbini 2016).

Starch granule morphology also contributes greatly to their resistance against digestive enzymes. Simpler and smaller granules are more susceptible to digestion than larger ones (Lehmann and Robin 2007; Zaman and Sarbini 2016).

8.2.2 Oligosaccharides

Oligosaccharides are short-chain polysaccharides containing up to 20 saccharide units, produced in the process of hydrolysis or enzymatic degeneration from larger chain polysaccharides. Among many available oligosaccharides, few are said to have prebiotic potential. Oligosaccharide prebiotics can be broadly divided into two major groups, e.g., inulin-type prebiotics and galactooligosaccharides (Kelly 2008). Other compounds may include lactulose, gluco-oligosaccharides, xylooligosaccharides, lactosucrose, Palatinose, etc.

8.2.3 Inulin-Type Prebiotics

The group “inulin-type prebiotics” consists of inulin, oligofructose, and fructooligosaccharides (FOS), linear chain oligosaccharide or polysaccharide primarily made up of fructose molecules linked by fructosyl-glucose or fructosyl-fructose linkages. Many vegetables like root veggies contain this group of prebiotic naturally.

Inulins are part of a larger group called “fructans”—a group of naturally occurring oligomer or polymer chain of fructose with most of its glycosidic bonds being fructosyl-fructose linkages (Roberfroid 2007; Kelly 2008; Guimarães et al. 2020). Many of these chains begin with fructose, while some may begin with a glucose unit showing a fructosyl-glucose linkage at the beginning. An inulin molecule having glucose at the beginning may be represented as GF_n, G being the glucose unit, F the fructose units, and n the number of fructose units (Kelly 2008). This linear chain of inulin is an α-D-glucopyranosyl-[β-D-fructofuranosyl]$_{n\text{-}1}$-β-D-fructofuranoside, and the fructosyl-glucose linkage in this case is always β (2↔1) similar to sucrose (Roberfroid 2007). On the other hand, an all-fructose inulin is designated as F_n, F being the fructose units and n being the number of them. Here, the all-fructose chain is β-D-fructopyranosyl-[β-D-fructofuranosyl]$_{n\text{-}1}$-β-D-fructofuranoside with β-(1↔2) linkage (Roberfroid 2007; Kelly 2008). The inulins having “n” number of fructose units also must have “n-1” fructosyl-fructose linkages between them. This number of fructose units (n) also represents the molecule's degree of polymerization (DP) (Kelly 2008). The presence of higher DP inulins results in more stable prebiotic foods (Guimarães et al. 2020). Commercially available inulin-type prebiotics are not of homogenous construction of a single type of inulin, but generally a complex blend of different inulin-type fructans having different DP.

8.2.4 Galactooligosaccharides

Galactooligosaccharides or more specifically transgalactooligosaccharides are products of transglycosylation of lactose. The product mixture usually contains oligosaccharides with three to five saccharide chains. Lactose is a disaccharide almost universally available in all mammalian milk in significant amounts.

Galactooligosaccharides (GOS) are produced as intermediate products of transgalactosylic hydrolysis of lactose by a hydrolase β-galactosidase that attacks the o-glucosyl group. According to Tzortzis and Vulevic (Tzortzis and Vulevic 2009), GOS can be defined as a mixture of by-products of lactose digestion with two to eight saccharide units and has a terminal glucose, and the rest of saccharides are galactose or disaccharides of galactose units. Plant GOS has lower prebiotic potential than animal origin GOS, as plant GOS is less selective in terms of bacterial fermentation, whereas milk GOS has shown to be selectively fermented mostly by bifidobacteria (Wilson and Whelan 2017). The activity of the saccharolytic enzyme β-galactosidase is much higher in bifidobacteria resulting in selective digestion of β-linked galactose. Thus, β-GOS specifically promotes the growth of bifidobacteria in vivo (Depeint et al. 2008; Tzortzis and Vulevic 2009).

Galactooligosaccharides are highly soluble, have natural sweetness, have good viscosity, and are very much stable at high temperatures or different pH levels. Moreover, GOS can be considered as dietary fibers. Their addition to food modulates food texture and fiber bulk. Commercially, these are extensively used in fermented milk products for their contribution to fiber content, inherent sweetness, or bifidogenic activities (Depeint et al. 2008).

8.2.5 Xylooligosaccharides

Xylooligosaccharides (XOS) are oligomers of xylose units produced from xylan containing lignocellulosic biomass by enzymatic or chemical hydrolysis or by their combination (Katapodis and Christakopoulos 2005; Samanta et al. 2015). XOS varies in structure like degree of polymerization or type of linkage depending upon the xylan sources. XOS are oligosaccharides of xylose units joined by β-(1 → 4) xylosidic linkages with number of xylose units varying between two and ten (Aachary and Prapulla 2011). XOS are heat- and acid-stable, making them well suited for prebiotic properties. They are alternative sweeteners having 92% relative sweetness to sucrose but without any blood sugar elevation. Other benefits include selective promotion of beneficial gut microflora growth, antioxidant properties, mild laxative properties, maintenance of serum lipid levels, etc. (Samanta et al. 2015).

8.2.6 Lactulose

Lactulose is a synthetic disaccharide produced from lactose by isomerization. It has a chemical structure of 4-*O*-β-D-galactopyranosyl-D-fructofuranose with fructose-galactose isomerization. It easily evades digestion in the stomach or intestine to reach the colon unaltered. It has laxative properties at a higher dose, while lower doses are prebiotic (Pranami et al. 2017). Lactulose does not occur naturally, but as its production involves heat treatment of milk, it may be present in heat-processed dairy products naturally (Olano and Corzo 2009). Lactulose being resistant to digestion is available to be selectively metabolized by the bifidobacteria and lactobacilli population of the hindgut producing short-chain fatty acids and increasing the fecal biomass (Ruszkowski and Witkowski 2019). On the other hand, upon lactulose administration, population of pathogenic groups like *Bacteroides* spp., *Streptococcus* spp., *Enterobacteriaceae*, *Clostridium* spp., and coliforms decreases along with carcinogenic and toxic microbial enzyme activities and metabolites (Tuohy et al. 2005). Lactulose also stimulates mineral absorption like that of calcium and magnesium (Olano and Corzo 2009).

8.2.7 Noncarbohydrate Oligosaccharides

Typically most prebiotics are carbohydrates of some kind. However, there are a few compounds such as cocoa-derived flavanols that are noncarbohydrate prebiotics as experiments have demonstrated its potential to stimulate lactic acid bacteria (Tzounis et al. 2011).

8.3 Prebiotics as Nutraceuticals

Nutraceutical is a conjugate word of "nutrition" and "pharmaceutical." These are food additives, derivatives, or functional foods known to provide additional health benefits to their original nutritional values (Mishra et al. 2018).

A broad range of food derivatives or functional foods comes under the umbrella of nutraceuticals including dietary fibers and prebiotics, probiotics, antioxidants, different spices, etc. (Das et al. 2012). Among these dietary fibers and prebiotics occupies a significant share among all nutraceuticals. Dietary fibers consist of insoluble dietary fibers such as celluloses, hemicelluloses, lignins, dextrins, or soluble dietary fibers that include pectins, β-glucans, etc. Soluble dietary fibers due to their property of contributing to viscosity and bulking create the feeling of fullness delaying gastric emptying and eventually modulate digestion (Leclere et al. 1994; Das et al. 2012). Dietary fibers also increase fecal mass by increasing water retention. Prolonged fermentation of the fibers also increases transit time leading to the promotion of bifidobacterial growth in the gut. Their benefits are limited not only to the dietary sector but also in other health-related aspects like lowering serum LDL, lowering hypertension or diabetes, or enhancing immunity (Montonen et al. 2003; Anderson et al. 2004; Watzl et al. 2005; Whelton et al. 2005; Das et al. 2012).

Short-chain nondigestible polysaccharides, which are prebiotics in their truest sense, are also a part of the nutraceutical family, as they provide several health benefits to the consumer along with their nutritional values. As discussed earlier, prebiotic consumption most significantly promotes the growth of gut microflora and eventually helps in metabolism (Manning and Gibson 2004; Depeint et al. 2008; Tzortzis and Vulevic 2009). Other health benefits include lactose tolerance, vitamin and mineral absorption, control of diabetes, blood lipid or blood pressure, improving digestion, reducing constipation, better immunity, etc. (Das et al. 2012).

There are ample references that support the health benefits of prebiotics in managing irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). Some studies have revealed that consumption of FOS and GOS has improved both of these signs (Moreau et al. 2003; Ewaschuk and Dieleman 2006; Whelan 2013; Wilson and Whelan 2017). However, there are some contrasting reports as well, which suggest rapid fermentation of oligosaccharides causes an increase in organic acids, which in turn damages mucous cells of the hindgut (Cherbut et al. 2003).

Studies have shown that inulin, FOS, and galactooligosaccharides have preventive effects against colorectal cancer by reducing enzymes responsible and carcinogens. Upon administration of oligosaccharide prebiotics, it has been shown that genotoxic enzymes like β-glucosidase, β-glucuronidase, and arylsulfatase are readily reduced (Wijnands et al. 2001; Hsu et al. 2004; Pool-Zobel 2005; Tuohy et al. 2005). One of the major end products of gut microbial fermentation of prebiotics, i.e., butyrates, also aids in maintaining gut epithelial health (Weng and Walker 2006). Studies have shown that butyrate can suppress NF-κB expression. Acetate on the other hand is known to elevate natural killer cells or antibody production in cancer patients (Macfarlane et al. 2008).

Studies have further revealed that nondigestible oligosaccharides that include our most common prebiotics like FOS, GOS, inulins, etc. are stimulatory to mineral absorption in the gut region (Scholz-Ahrens et al. 2001; Scholz-Ahrens et al. 2007). Absorption of minerals such as Ca, Mg, etc. has been positively increased in the presence of different prebiotic components (Scholz-Ahrens et al. 2001). Inulin-type fructans are reported to aid in preventing Mg deficiency in adults (Scholz-Ahrens et al. 2007).

Although the mechanism is not exactly known, it has been seen that the consumption of prebiotics helps in lipid regulation. Production of propionate from fermentation of FOS reduces blood lipid contents by inhibiting lipidogenic enzymes in the liver (Bornet et al. 2002). Interestingly, however, it was seen in the same study that this anti-lipidogenic property was only seen in the case of individuals with diabetes or other such conditions causing hyperlipidemia, but not in healthy individuals (Bornet et al. 2002). Propionate, an oligosaccharide fermentation by-product, on reaching the liver, inhibits HMG-CoA reductase stopping cholesterol pathways (Levrat et al. 1994).

Evidences from animal studies showed inulins helped in reducing plasma VLDL triglyceride concentration (Fiordaliso et al. 1995). In human models, inulins are more effective than FOS in this respect of reducing cholesterol or plasma lipids. According to Vanhoof and De Schrijver (1995), the reduction in cecal pH brought about by the fermentation of prebiotics reduces available soluble bile acids, which in turn decreases lipid absorption. This same study also suggested that increased upper large intestine decreases lipid and cholesterol reabsorption, increases cholesterol metabolism in the liver, and thus finally decreases the plasma lipid content (Vanhoof and De Schrijver 1995).

8.4 Role of Prebiotics in Modulating Host-Gut Microbiota Interactions

The large intestine is the most metabolically active organ within the body. Not only the gut itself but the astounding and diverse microbial population that resides within is also very much metabolically active. The large interface along the inner wall of the large intestine provides ample space for hosting a large population of almost more than 1000 species of different microorganisms, whose collective number surpasses the total innate cells of the host's body by more than ten times (Shanahan 2002). Thus, the large intestine is no more considered as an organ merely for the absorption of digested foods or storage of undigested ones. The resident microbial community of the gut itself forms an organ performing several functions along with the designated ones of the large intestine (O'Hara and Shanahan 2006).

Due to variability in pH, availability of nutrients or oxygen, or difference in transit time, the gut microbe population is not uniform throughout the whole length of the GI tract, rather highly concentrated in the large intestine and colon. A favorable physicochemical environment that includes slow transit rate, availability of ample amount of fermentable undigested food, anaerobic condition, and perfect pH aids to the settlement of the microbe community there (Macfarlane et al. 2006; Drakoularakou et al. 2010).

There is a complex ecological system of microbiota residing in the gut, some of which are beneficial, some being benign, but few are harmful to the host's health (Gibson 2008). This complexity leads to homeostasis of the community, and in turn, they also form a barrier against any infecting pathogens to a significant extent. Pathogenic microbial strains that reside in the gut are extensively studied. *Helicobacter pylori* is the most prevalent pathogenic bacteria throughout the world (Khalifa et al. 2010). Other microbes include strains of *Escherichia coli*, *Clostridium difficile*, and *Salmonella* spp. (Tuohy et al. 2005). Two groups of microbes, the bifidobacteria and lactobacilli, are major residents of the large intestine that directly benefit the hosts' health (Tuohy et al. 2005). Among the different strategies to bolster the population and diversity of beneficial gut microflora, the administration of prebiotics is one of the more recent and effective approaches. It is a complementary approach that stimulates the growth of indigenous bifidobacteria and lactobacilli populations of the host.

Prebiotic components in food increase the available resources for the bifidobacteria and lactobacilli to ferment, eventually, favoring the increase of their population. Moreover, an increase in the population size of these microbes outcompetes and reduces the population size of other pathogenic strains that benefit the host in two front manners. For example, it is well studied and established that in ideal pH and anaerobic conditions, FOS and inulins not only stimulated the growth of *Bifidobacterium infantis* but also resisted the growth of *Escherichia coli* and *Clostridium perfringens* (Nitzan et al. 2016). Consumption of FOS and inulins in adults showed significant bifidogenic activity and also reduced populations of pathogenic bacteria as revealed from the study of stool (Gibson and Roberfroid 1995; Roberfroid 2007). Due to the presence of β-fructofuranosidase enzyme, bifidobacteria are capable of fermenting and generating energy from fructans (Imamura et al. 1994).

Similarly, in as early as 1983, the prebiotic benefits of GOS were demonstrated in monoculture experiments, where the presence of GOS stimulated several species of *Bifidobacterium* and *Lactobacillus* (Tanaka et al. 1983). Fermentation of GOS by bifidobacteria decreases gut pH by producing short-chain fatty acids (SCFAs) and other organic acids (Angus et al. 2005). SCFAs are readily absorbed by different groups of cells, like butyrate by colonocytes, acetate by peripheral tissues, or propionate by the liver. Butyrates are also known for their protective effect on colon cancer and colitis. Thus, an increase in the production of SCFAs has a protective effect on colon health. The acetate and propionate ratio also maintains the serum lipid level (Wong et al. 2006). GOS has been shown to have bifidogenic effects in rats with human-associated gut microflora (Klinder et al. 2004). Similar to FOS, inulins, and GOS, resistant starches are also proved to increase production of SCFA, assisting in better colon health (Wong et al. 2006).

Reports also suggest that oligosaccharides with mannose side chains block *E. coli* and other pathogenic microbes from adhesion to the mucosal wall of the gut (Shoaf-Sweeney and Hutkins 2008). The application and usefulness of prebiotic in maintaining the gut environment are depicted in Fig. 8.1.

8.5 Therapeutic Role of Prebiotics in Various Inflammatory and Infectious Diseases of the Gut

Prebiotics are nowadays widely used in various food and vitamin supplements and have been used to enhance the immunity and as complementary medicine supporting therapeutic potential against various inflammatory diseases associated with the gut, including inflammatory bowel disease (IBD), ulcerative colitis (UC), and gastritis, and most recently discovered against various cancers associated with the digestive system and also for various infectious diseases of the gut. Furthermore, it is also very much essential in decreasing the complications associated with lactose intolerance and susceptibility to allergies and enhances the bioavailability of essential probiotics associated with nutrient absorption and digestion (Ahmad and Khalid 2018). The widely used prebiotics as therapeutic agents are the short-chain carbohydrates that include the mannan-oligosaccharides, galactoglucomannans, oligofructose, inulin, lactose, etc. and work principally by increasing the beneficial microbiota in the gut

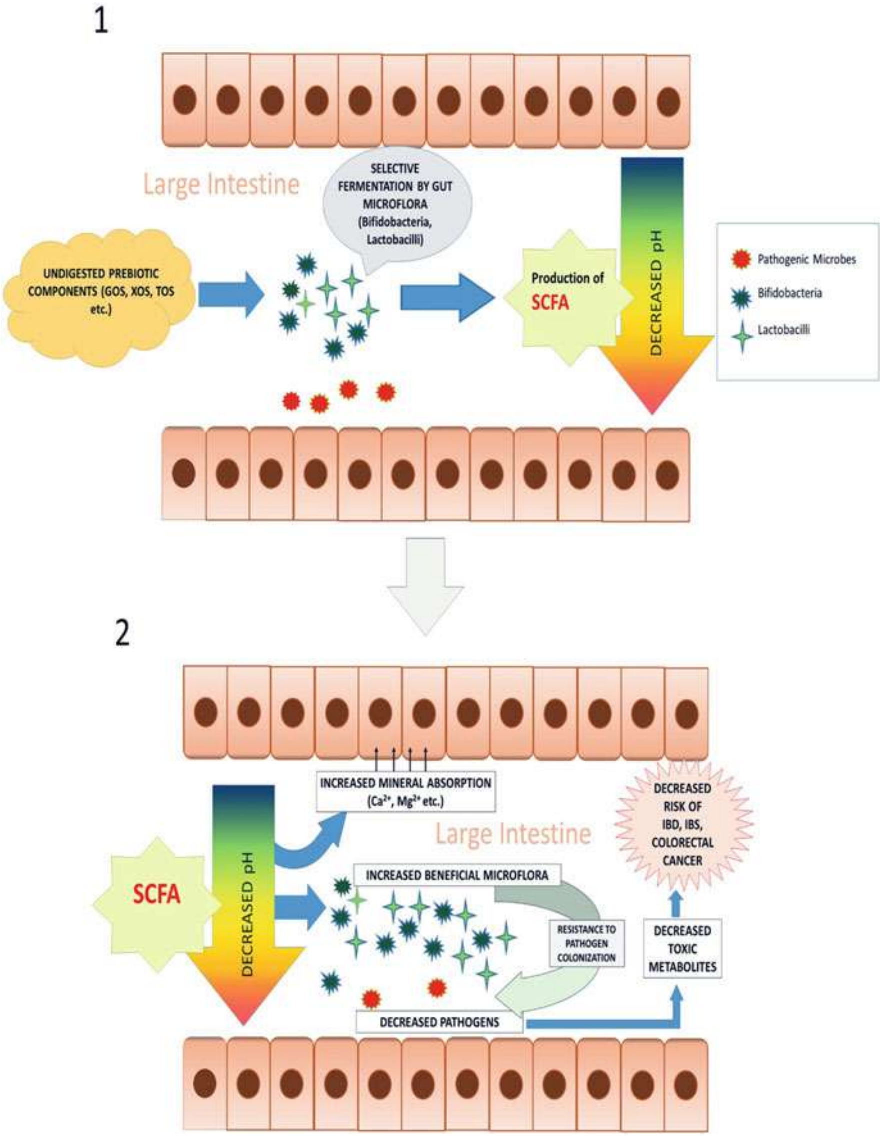


Fig. 8.1 Applications and usefulness of prebiotics in maintaining and improving the gut microenvironment

like *Bifidobacterium* and *Lactobacillus* and giving rise to SCFA to reduce the inflammation associated with the gut (Macfarlane and Gibson 1997). The use of prebiotics as a therapeutic agent against the amelioration of various inflammatory and infectious diseases is illustrated in Fig. 8.2.

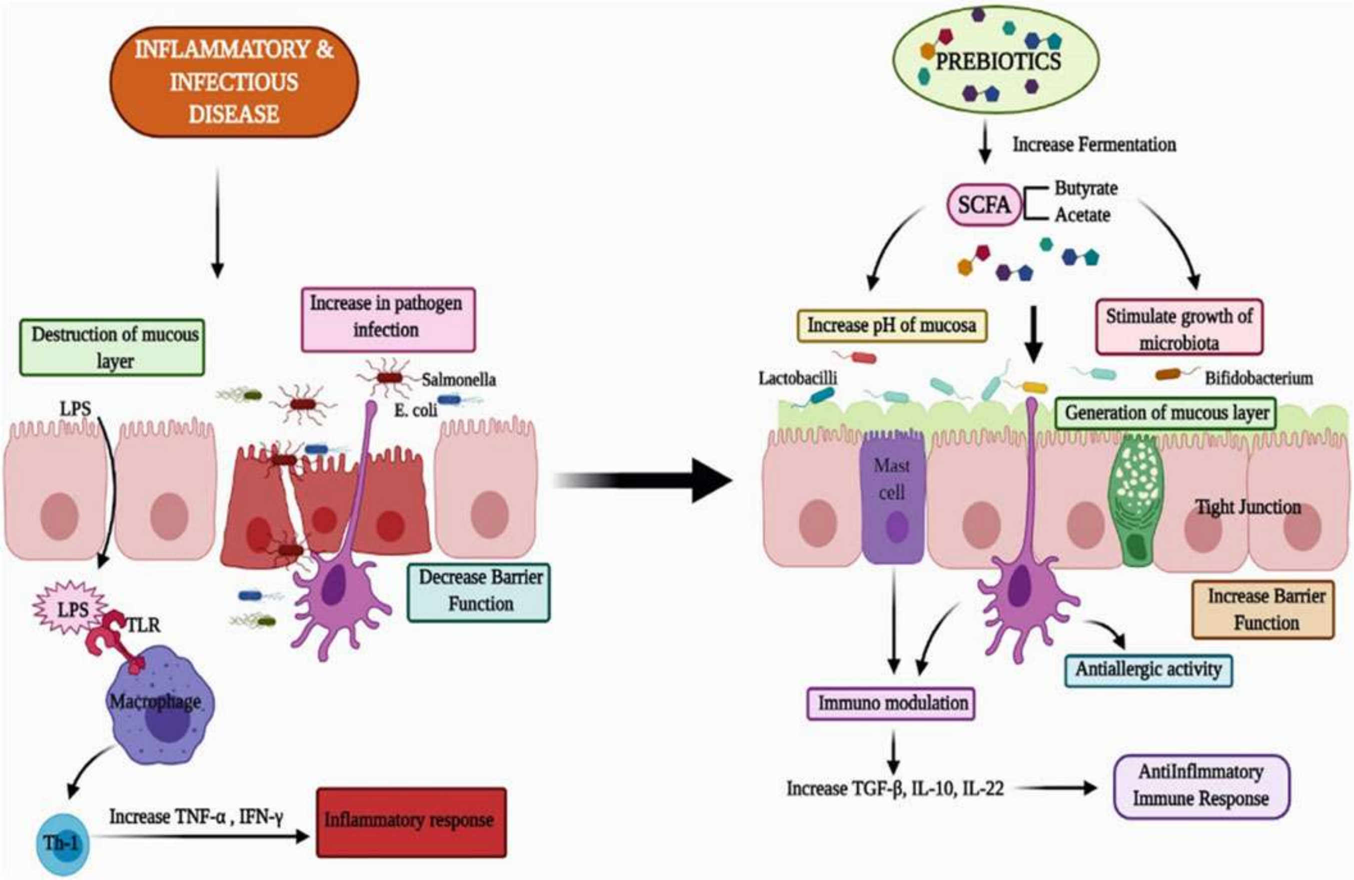


Fig. 8.2 Therapeutic role of prebiotics against the inflammatory and infectious diseases of the gut

It is often considered as dietary fiber like β-glucan that enhances the bioavailability of nutrients and minerals and supports the growth of probiotics and microbiota. The various prebiotics with their mechanism of action as therapeutic are demonstrated in Table 8.1.

8.5.1 Mechanism of Action at the Cellular and Molecular Level for Inflammatory Diseases of the Gut

The basic principle of action of prebiotics in various gut-related inflammatory diseases is based on stimulating the growth of beneficial gut microbiota, fermentation leading to the production of SCFA, increasing the absorption of micronutrients, modification of xenobiotic metabolic enzymes, and modulation of gene expression and immune responses (Ahmad and Khalid 2018). The dietary fibers act as a prebiotic and mainly work as anti-inflammatory compounds curing the consequences associated with IDB and UC. It mainly increases the concentration of SCFA, such as acetate, butyrate, and propionate, to induce the immune response and effectively diminish the inflammation (Vieira et al. 2013). SCFA also decreases the luminal pH of the colon, making it inhabitable to the pathogenic bacteria, and reduces their enzymatic activity (Looijer-Van Langen and Dieleman 2009). Butyrate is the most common and important type of SCFA. It works as an energy source that is transported to the epithelium cells of the colon through the monocarboxylate transporters, like MCT-1107, helps in proliferating the cells, and acts as a barrier to decrease oxidative stress and DNA damage (Gibson et al. 1999; Vieira et al. 2013; Iriti and Varoni 2014). The release of pro-inflammatory cytokines like TNF-α and IFN-γ increases the permeability of the epithelial layer of the gut leading to the disruption of the epithelial barrier and is the main cause of pathogenesis of IBD (Kucharzik et al. 2006). It is found that with the increase in the production of butyrate, the inflammation decreases by the induction in the concentration of T-regulatory (T-reg) and inversely decreases INF-γ (Klampfer et al. 2003). Furthermore, it also triggers the activation of various nuclear transcription factors and peroxisome proliferator activator receptor-γ (PPARy) that leads to the reduction of pro-inflammatory pathways including NF-kB and STAT pathways, resulting in suppression of gut inflammation (Su et al. 1999). In the case of colon cancer, it is found that butyrate affects the binding of DNA and transcription by inhibiting histone deacetylases (HDACs) (Davie 2003). It reduces the survival of tumor cells by induction of apoptosis and modulation of genes associated with the regulation of metabolic and oxidative stress and further induces glutathione *S*-transferases in the colon tissue cell to detoxify the dietary carcinogens (Macfarlane 2010). The induction of a high concentration of butyrate within the colon is directed by the production of short-chain fructooligosaccharides. This shows a surge in the mucosal crypt height and density of epithelial cells in neonatal pigs, and its prolonged effects alter the fermentative activity within the colon (Bouhnik et al. 1997; Looijer-Van Langen and Dieleman 2009).

Table 8.1 Therapeutic action of various prebiotics against gut-associated diseases

Types of prebiotics	Structure	Therapeutic mechanism of action	References
Fructans (inulin and Fructooligosaccharides (FOS))	Fructosyl-fructose β (2X1) glycosidic bonds	Increase production of SCFA and *Lactobacil*lus and *Bifidobacterium* species within gut, act against ROS, stimulate antioxidative enzymes, and reduce oxidative stress, and TNF-α and IL-1α in UC patients; TLR-mediated immune response are mediated	Furrie et al. (2005), Pasqualetti et al. (2014), Holscher (2017)
Lactulose	Synthetic disaccharide galactose-fructose β (1–4)-linked	At lower concentrations, regulate *Bifidobacterium* growth and SCFA production; higher rate results in increase acetate production	Bothe et al. (2017)
Galactooligosaccharides (GOS)	Galactose polymer with a terminal β-linked Glucose monomer	Increase *Bifidobacterium* growth, immunomodulation by increasing concentration of IL-10, IL-8, NK cells, C-reactive proteins and decrease in IL-1β, TNF-α; lipid peroxidation	Vulevic et al. (2015)
Xylooligosaccharides (XOS)	Xylose units linked by β(1–4) bonds	Increase growth of *Bifidobacterium lactis*, *Bifidobacterium adolescentis*, and *Lactobacillus* Decrease *Clostridium* growth	Lin et al. (2016)
Arabinooligosaccharides (AOS)	α (1–6)-linked backbone of *L. arabinosyl* residues, either be single- or double-substituted with α(1–2)- and/or α(1–3)-linked L-arabinosyl residues	Increase the growth of *Lactobacillus* and *Bifidobacterium*; decreases the growth of *Firmicutes*, *Bacteroidetes*, and *Desulfovibrio*; lower gut pH; increase production of acetate; and ameliorate inflammatory responses in UC	Vigsnæs et al. (2011)

A study by Kanner et al. (2001) suggested that the production of gastric acid induces the oxidation of lipid and food substances and the dietary antioxidants like inulin prevent lipid peroxidation within the stomach and protect from oxidative stress (Kanner and Lapidot 2001). Fructans, such as inulin, are dietary fibers that stimulate gastrointestinal (GI) motility to induce antioxidative activities. This in turn, protect the colon mucosa from LPS-induced oxidative stress and act as a therapeutic agent (Pasqualetti et al. 2014). Lactulose is used as a functional food ingredient and also as a laxative or ammonia-lowering drug that helps in enhancing and regulating the growth of *Bifidobacterium* and *Lactobacillus* based on the concentration of dose and duration of application; 2 g induces the SCFA production, while 5 g resulted in the growth of bacterium and decrease in concentration of butyrate and enhancement in acetate (Bothe et al. 2017).

Furrie et al. (2005) tested with 12 g of oligofructose-enriched inulin and capsules containing 2×10^{11} freeze-dried *Bifidobacterium longum* on active UC patients and found a 42-fold increase in bifidobacteria growth on the rectal mucosa and also a diminution of TNF-α and IL-1α and significant reduction in the mRNA levels for human β defensins 2, 3, and 4 (Furrie et al. 2005). The application of FOS, GOS, and inulin in the colitis induced animal model showed a reduction of mucosal inflammation; also, it increases SCFA concentration, induces the growth of lactobacilli and bifidobacteria, and mitigates NF-kB pathway activation and proliferation (Kanauchi et al. 2003). The application of various types of prebiotics against various inflammatory diseases associated with the gut is listed in Table 8.2.

8.5.2 Mechanism of Action at the Cellular and Molecular Level for Infectious Diseases of the Gut

Apart from the therapeutic effects against various inflammatory diseases of the gut and its beneficial effects on inducing the growth of lactobacilli and bifidobacteria, prebiotics play a vital role in the treatment and amelioration of various infectious diseases of the gut. It mainly works by highly activating the immune system for defense against the pathogenic microbes that enter the gut. The increase in SCFA, and lowering in pH of the gut degrades the enzymes of pathogens to maintain the proper microenvironment to fight against the infectious agents. Several studies on animal model have shown the use of prebiotics as therapeutic against various pathogenic microbes including *Salmonella*, *Escherichia coli*, and *Listeria monocytogenes.* It has been studied that prebiotics in association with probiotics and other beneficial gut microbiota play a prominent role in the alleviation of infection, and it has been found that the ingestion of a combination of FOS or inulin with calcium elevates the translocation of *Salmonella* to the extra-intestinal site of rat model. It is suggested that prebiotic induced acidification of the intestine, making it permeable to the pathogenic bacteria, which can be counteracted by the use of calcium (Bovee-Oudenhoven et al. 1997). In humans, the regular use of 48% concentration of GOS indicated the lower duration of travelers' diarrhea while traveling across highly prone area to intestinal infection (Drakoularakou et al.

Table 8.2 Application of various prebiotics against various inflammatory diseases of the gut

Disease	Types of prebiotics	Mechanism of action	References
Inflammatory bowel disease (IBD)	Inulin-type fructans	It helps mesalazine to alleviate intestinal inflammation	Joossens et al. (2011)
	Mixture of FOS And GOS	Improvement in gastric emptying and bowel motility	Indrio et al. (2009)
Ulcerative colitis (UC)	Arabinooligosaccharides (AOS)	Stimulation of bacteria eliciting anti-inflammatory responses and production of acetate	Vigsnæs et al. (2011)
	Oligofructose-enriched inulin	Increase in the growth of bifidobacteria and reduction of TNF-α and IL-1α and significant reduction in the mRNA levels for human β defensins 2, 3, and 4	Furrie et al. (2005)
	Germinated barley foodstuff (GBF)	Prevents remission of mild-to-moderate active UC	Bamba et al. (2002)
Colon cancer	Inulin-enriched FOS	Cell growth inhibition thus decreases the progression of colorectal cancer	Klinder et al. (2004)
	Inulin-type fructans	Enhanced the *Bifidobacterium* count, lowered pH, immune modulation, decreased colon neoplasms by induction of azoxymethane	Williams et al. (2007)
	AXOS-supplemented diet	Reduced the rate of preneoplastic wounds	Femia et al. (2010)

2010). Many studies suggested that eating of diet supplement with FOS reduces the risk of relapsing of bacteria-caused diarrhea and susceptibility to infectious disease (Lewis et al. 2005). It is being found that several milk-derived oligosaccharide prebiotics structurally identical to the saccharide containing glycoproteins act as receptor analogs and bind with pathogenic cells; as a result, the pathogenic cell is unable to bind with the host cell receptor, thereby inhibiting the infection (Kunz et al. 2000).

8.6 Therapeutic Promises of Prebiotics

The treatment of inflammatory disease depends on the use of corticosteroids, aminosalicylates, or immunomodulatory agents based on the severity and type of disease, which work upon by suppression and modulation of the host immune system against the inflammatory responses (Macfarlane 2010). However, prolonged use of these medicines resulted in intolerance and side effects (Navarro and Hanauer

2003). Antibiotics have been used for the treatment of IBD (including CD and UC), septic complications associated with IBD, and regulating bacterial overgrowth and also help in preventing remission of IBD (Nitzan et al. 2016). However, the excessive use of antibiotics resulted in resistance toward the bacteria and even disrupts gut microbiota. The prolonged and frequent use of metronidazole leads to gastrointestinal disorders and may even cause permanent peripheral neuropathy (Sarna et al. 2013). Nonsteroidal anti-inflammatory drugs (NSAIDS) are widely used medications used against inflammatory responses; however, their extensive use leads to the formation of gastrointestinal toxicity including mucosal injury resulting in erosions, ulcers, colonic bleeding, and bowel disorders (Wolfe et al. 1999). It can interfere with the cyclooxygenase (COX) pathways resulting in the generation of prostanoids (prostaglandins, prostacyclin, and thromboxane) that leads to reduction of effectiveness of the mucus-bicarbonate barrier, gastric acid, and other enzymes causing damage to the gut (Russell 2001).

The concept of prebiotic is introduced by Gibson and Roberfroid (1995); however, in this modern times, it has become an essential therapeutic tool for treating various inflammatory and infectious diseases associated with the gut by substantially increasing the growth of beneficial microbes inside the gut, permitting their modifications, and enhancing their ability against the disease (Sun and Chang 2014). The usefulness of the prebiotics in various inflammatory and infectious diseases for the gut is already discussed in the above sections. Moreover, the association of prebiotics with the probiotics in the regular diet seems to be beneficial for the human gut.

8.7 Prospects and Challenges

Worldwide research on prebiotics is a very important area of research, and there are various guidelines regarding the use of it. It has been shown to have a very beneficial role in the process of digestion, protection against disease, and increasing immune response and as therapeutics. However, there are still many lacuna with the knowledge of how exactly the prebiotics work and the action of molecular mechanism during its intervention on various metabolic pathways (Mishra et al. 2018). The use of dietary fiber in food shows various essential and vital results; however, various dietary fibers show adverse effect on the health, and it is also to be noted that all prebiotics are fibers, but all fibers are not prebiotics due to their variation in structure and functions (Biswal et al. 2017). Prebiotic safety is very much essential, as many of the prebiotics show moderate to severe side effects. The intestinal enzymes cannot breakdown the oligosaccharides and polysaccharides and thus are transported to the colon where it is fermented by the microbiota. This results in osmotic functions including osmotic diarrhea, bloating, and flatulence due to the overuse of prebiotics (Davani-Davari et al. 2019).

8.8 Conclusion and Future Directions

The use of prebiotics is shown to be very much essential in the daily diet. Prebiotics, not only increases the bioavailability of the essential minerals within the food but also increases its bioabsorption and are used as therapeutic against various diseases associated with the gut. It consequently enhances the growth of beneficial microbiota of the gut and thus induction in the process of digestion. According to the dietary guidelines, it is included that the use of prebiotic ingredients in the food inherited nutritive values and enhances the immune and digestive response and permitted for daily intake.

This study is to focus on the area of prebiotics that can be used as nutraceuticals, as food diet, and their therapeutic potentials. The biochemical properties of the various prebiotics are available for the use as nutraceutical in human diet. It further enlightens the association between the prebiotics and the gut microbiota. It has been found that the use of nutraceutical enhances the growth of the various bacteria. It specially increases the numbers of lactobacilli and bifidobacteria. The use of prebiotics subsequently helps in lowering the inflammation associated with IBD including CD and UC and also helps in inhibiting the progression of colon cancer. Furthermore, it is also very much useful in protection from the infectious pathogens entering the gut. However, many area of research regarding the actual mechanism of action of prebiotic for altering the metabolic pathways are still undiscovered and still to overcome the consequences of side effect of excessive or prolonged use. Apart from these, prebiotics are very much essential and beneficial as therapeutics and must be used along with diet.

Acknowledgments We have incorporated certain number of references due to the limitation in space with having a prodigious respect to all the uncited related articles. Ritwik Patra thanks the Department of Higher Education, Government of West Bengal, for awarding Swami Vivekananda Merit-cum-Means Fellowship. We acknowledge the use of BioRender app for the preparation of illustrations.

References

Aachary AA, Prapulla SG (2011) Xylooligosaccharides (XOS) as an emerging prebiotic: microbial synthesis, utilization, structural characterization, bioactive properties, and applications. Compr Rev Food Sci Food Saf 10:2–16. https://doi.org/10.1111/j.1541-4337.2010.00135.x

Ahmad A, Khalid S (2018) Chapter 3 - Therapeutic aspects of probiotics and prebiotics. In: Holban AM, Grumezescu AM Diet, Microbiome and health. Handbook of Food Bioengineering. Academic Press Boston, pp. 53–91 https://doi.org/10.1016/B978-0-12-811440-7.00003-X

Anderson JW, Randles KM, Kendall CWC, Jenkins DJA (2004) Carbohydrate and fiber recommendations for individuals with diabetes: a quantitative assessment and meta-analysis of the evidence. J Am Coll Nutr 23:5–17. https://doi.org/10.1080/07315724.2004.10719338

Angus F, Smart S, Shortt C (2005) Prebiotic ingredients with emphasis on galacto-oligosaccharides and fructo-oligosaccharides. Probiotic Dairy Prod 18:120–137

Bamba T, Kanauchi O, Andoh A, Fujiyama Y (2002) A new prebiotic from germinated barley for nutraceutical treatment of ulcerative colitis. J Gastroenterol Hepatol 17:818–824. https://doi.org/10.1046/j.1440-1746.2002.02709.x

Biswal P, Pal A, Das AP (2017) Current trends and future prospective of prebiotics as therapeutic food. In: Microbial production of food ingredients and additives. Elsevier, Amsterdam, pp 57–88. https://doi.org/10.1016/B978-0-12-811520-6.00003-9

Bornet FRJ, Brouns F, Tashiro Y, Duvillier V (2002) Nutritional aspects of short-chain fructooligosaccharides: natural occurrence, chemistry, physiology and health implications. Dig Liver Dis 34:S111–S120. https://doi.org/10.1016/S1590-8658(02)80177-3

Bothe MK, Maathuis AJH, Bellmann S, Van der Vossen JMBM, Berressem D, Koehler A, Schwejda-Guettes S, Gaigg B, Kuchinka-Koch A, Stover JF (2017) Dose-dependent prebiotic effect of lactulose in a computer-controlled in vitro model of the human large intestine. Nutrients 9:767. https://doi.org/10.3390/nu9070767

Bouhnik Y, Flourié B, D'Agay-Abensour L, Pochart P, Gramet G, Durand M, Rambaud J-C (1997) Administration of transgalacto-oligosaccharides increases fecal bifidobacteria and modifies colonic fermentation metabolism in healthy humans. J Nutr 127:444–448. https://doi.org/10.1093/jn/127.3.444

Bovee-Oudenhoven IM, Termont DS, Heidt PJ, Van der Meer R (1997) Increasing the intestinal resistance of rats to the invasive pathogen Salmonella enteritidis: additive effects of dietary lactulose and calcium. Gut 40:497–504. https://doi.org/10.1136/gut.40.4.497

Cherbut C, Michel C, Lecannu G (2003) The prebiotic characteristics of fructooligosaccharides are necessary for reduction of TNBS-induced colitis in rats. J Nutr 133:21–27. https://doi.org/10.1093/jn/133.1.21

Cummings JH, Macfarlane GT (1991) The control and consequences of bacterial fermentation in the human colon. J Appl Bacteriol 70:443–459. https://doi.org/10.1111/j.1365-2672.1991.tb02739.x

Das L, Bhaumik E, Raychaudhuri U, Chakraborty R (2012) Role of nutraceuticals in human health. J Food Sci Technol 49:173–183. https://doi.org/10.1007/s13197-011-0269-4

Davani-Davari D, Negahdaripour M, Karimzadeh I, Seifan M, Mohkam M, Masoumi SJ, Berenjian A, Ghasemi Y (2019) Prebiotics: definition, types, sources, mechanisms, and clinical applications. Foods 8:92. https://doi.org/10.3390/foods8030092

Davie JR (2003) Inhibition of histone deacetylase activity by butyrate. J Nutr 133:2485S–2493S. https://doi.org/10.1093/jn/133.7.2485S

Depeint F, Tzortzis G, Vulevic J, I'Anson K, Gibson GR (2008) Prebiotic evaluation of a novel galactooligosaccharide mixture produced by the enzymatic activity of Bifidobacterium bifidum NCIMB 41171, in healthy humans: a randomized, double-blind, crossover, placebo-controlled intervention study. Am J Clin Nutr 87:785–791. https://doi.org/10.1093/ajcn/87.3.785

Drakoularakou A, Tzortzis G, Rastall RA, Gibson GR (2010) A double-blind, placebo-controlled, randomized human study assessing the capacity of a novel galacto-oligosaccharide mixture in reducing travellers' diarrhoea. Eur J Clin Nutr 64:146–152. https://doi.org/10.1038/ejcn.2009.120

Englyst KN, Liu S, Englyst HN (2007) Nutritional characterization and measurement of dietary carbohydrates. Eur J Clin Nutr 61:S19–S39. https://doi.org/10.1038/sj.ejcn.1602937

Ewaschuk JB, Dieleman LA (2006) Probiotics and prebiotics in chronic inflammatory bowel diseases. World J Gastroenterol 12(37):5941–5950. https://doi.org/10.3748/wjg.v12.i37.5941

Femia AP, Salvadori M, Broekaert WF, François IEJA, Delcour JA, Courtin CM, Caderni G (2010) Arabinoxylan-oligosaccharides (AXOS) reduce preneoplastic lesions in the colon of rats treated with 1, 2-dimethylhydrazine (DMH). Eur J Nutr 49:127–132. https://doi.org/10.1007/s00394-009-0050-x

Fiordaliso M, Kok N, Desager J, Goethals F, Deboyser D, Roberfroid M, Delzenne N (1995) Dietary oligofructose lowers triglycerides, phospholipids and cholesterol in serum and very low density lipoproteins of rats. Lipids 30:163–167. https://doi.org/10.1007/BF02538270

Fuentes-Zaragoza E, Sánchez-Zapata E, Sendra E, Sayas E, Navarro C, Fernández-López J, Pérez-Alvarez JA (2011) Resistant starch as prebiotic: a review. Starch 63:406–415

Furrie E, Macfarlane S, Kennedy A, Cummings JH, Walsh SV, O'Neil DA, Macfarlane GT (2005) Synbiotic therapy (Bifidobacterium longum/synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. Gut 54:242–249

Gibson GR (1998) Dietary modulation of the human gut microflora using prebiotics. Br J Nutr 80: S209–S212. https://doi.org/10.1017/S0007114500006048

Gibson GR (2008) Prebiotics as gut microflora management tools. J Clin Gastroenterol 42:S75–S79. https://doi.org/10.1097/MCG.0b013e31815ed097

Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125:1401–1412. https://doi.org/10.1093/jn/125.6.1401

Gibson PR, Rosella O, Wilson AJ, Mariadason JM, Rickard K, Byron K, Barkla DH (1999) Colonic epithelial cell activation and the paradoxical effects of butyrate. Carcinogenesis 20:539–544. https://doi.org/10.1093/carcin/20.4.539

Guimarães JT, Silva EK, Arruda HS, Freitas MQ, Pastore GM, Meireles MAA, Cruz AG (2020) How does the degree of inulin polymerization affect the bioaccessibility of bioactive compounds from soursop whey beverage during in vitro gastrointestinal digestion? Food Hydrocoll 101:105511

Holscher HD (2017) Dietary fiber and prebiotics and the gastrointestinal microbiota. Gut Microbes 8:172–184. https://doi.org/10.1080/19490976.2017.1290756

Hsu C-K, Liao J-W, Chung Y-C, Hsieh C-P, Chan Y-C (2004) Xylooligosaccharides and fructooligosaccharides affect the intestinal microbiota and precancerous colonic lesion development in rats. J Nutr 134:1523–1528. https://doi.org/10.1093/jn/134.6.1523

Imamura L, Hisamitsu K, Kobashi K (1994) Purification and characterization of β-fructofuranosidase from Bifidobacterium infantis. Biol Pharm Bull 17:596–602. https://doi.org/10.1248/bpb.17.596

Indrio F, Riezzo G, Raimondi F, Francavilla R, Montagna O, Valenzano ML, Cavallo L, Boehm G (2009) Prebiotics improve gastric motility and gastric electrical activity in preterm newborns. J Pediatr Gastroenterol Nutr 49:258–261. https://doi.org/10.1097/MPG.0b013e3181926aec

Iriti M, Varoni EM (2014) Cardioprotective effects of moderate red wine consumption: polyphenols vs. ethanol. J Appl Biomed 12:193–202. https://doi.org/10.1016/j.jab.2014.09.003

Joossens M, Huys G, Van Steen K, Cnockaert M, Vermeire S, Rutgeerts P, Verbeke K, Vandamme P, De Preter V (2011) High-throughput method for comparative analysis of denaturing gradient gel electrophoresis profiles from human fecal samples reveals significant increases in two bifidobacterial species after inulin-type prebiotic intake. FEMS Microbiol Ecol 75:343–349. https://doi.org/10.1111/j.1574-6941.2010.01008.x

Kanauchi O, Mitsuyama K, Araki Y, Andoh A (2003) Modification of intestinal flora in the treatment of inflammatory bowel disease. Curr Pharm Des 9:333–346. https://doi.org/10.2174/1381612033391883

Kanner J, Lapidot T (2001) The stomach as a bioreactor: dietary lipid peroxidation in the gastric fluid and the effects of plant-derived antioxidants. Free Radic Biol Med 31:1388–1395. https://doi.org/10.1016/S0891-5849(01)00718-3

Katapodis P, Christakopoulos P (2005) Xylanases as a tool for the production of novel phytopharmaceuticals. NutraCos 4:17–21

Kelly G (2008) Inulin-type prebiotics--a review: part 1. Altern Med Rev 13:315

Khalifa MM, Sharaf RR, Aziz RK (2010) Helicobacter pylori: a poor man's gut pathogen? Gut Pathog 2:2

Klampfer L, Huang J, Sasazuki T, Shirasawa S, Augenlicht L (2003) Inhibition of interferon γ signaling by the short chain fatty acid Butyrate11Montefiore 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 P3. Mol Cancer Res 1:855–862

Klinder A, Gietl E, Hughes R, Jonkers N, Karlsson P, McGlyn H, Pistoli S, Tuohy K, Rafter J, Rowland I (2004) Gut fermentation products of insulin-derived prebiotics beneficially modulate markers of tumour progression in human colon tumour cells. Int J Canc Prev 1:19–32

Kucharzik T, Maaser C, Lügering A, Kagnoff M, Mayer L, Targan S, Domschke W (2006) Recent understanding of IBD pathogenesis: implications for future therapies. Inflamm Bowel Dis 12: 1068–1083. https://doi.org/10.1097/01.mib.0000235827.21778.d5

Kunz C, Rudloff S, Baier W, Klein N, Strobel S (2000) Oligosaccharides in human milk: structural, functional, and metabolic aspects. Annu Rev Nutr 20:699–722. https://doi.org/10.1146/annurev.nutr.20.1.699

Leclere CJ, Champ M, Boillot J, Guille G, Lecannu G, Molis C, Bornet F, Krempf M, Delort-Laval J, Galmiche JP (1994) Role of viscous guar gums in lowering the glycemic response after a solid meal. Am J Clin Nutr 59:914–921. https://doi.org/10.1093/ajcn/59.4.914

Lee B, Bello-Pérez LA, Lin AH, Kim CY, Hamaker BR (2013) Importance of location of digestion and colonic fermentation of starch related to its quality. Cereal Chem 90:335–343. https://doi.org/10.1094/CCHEM-05-13-0095-FI

Lehmann U, Robin F (2007) Slowly digestible starch–its structure and health implications: a review. Trends Food Sci Technol 18:346–355. https://doi.org/10.1016/j.tifs.2007.02.009

Levrat M-A, Favier M-L, Moundras C, Rémésy C, Demigné C, Morand C (1994) Role of dietary propionic acid and bile acid excretion in the hypocholesterolemic effects of oligosaccharides in rats. J Nutr 124:531–538. https://doi.org/10.1093/jn/124.4.531

Lewis S, Burmeister S, Brazier J (2005) Effect of the prebiotic oligofructose on relapse of Clostridium difficile-associated diarrhea: a randomized, controlled study. Clin Gastroenterol Hepatol 3:442–448. https://doi.org/10.1016/S1542-3565(04)00677-9

Lin S-H, Chou L-M, Chien Y-W, Chang J-S, Lin C-I (2016) Prebiotic effects of xylooligosaccharides on the improvement of microbiota balance in human subjects. Gastroenterol Res Pract 2016:5789232. https://doi.org/10.1155/2016/5789232

Looijer-Van Langen MAC, Dieleman LA (2009) Prebiotics in chronic intestinal inflammation. Inflamm Bowel Dis 15:454–462. https://doi.org/10.1002/ibd.20737

Macfarlane S (2010) Chapter 10 – Prebiotics in the gastrointestinal tract. In: Watson RR, Preedy V (eds) Bioactive foods in promoting health. Probiotics and prebiotics. Academic Press, Boston, pp 145–156. https://doi.org/10.1016/B978-0-12-374938-3.00010-4

Macfarlane GT, Gibson GR (1997) Carbohydrate fermentation, energy transduction and gas metabolism in the human large intestine. In: Gastrointestinal microbiology. Springer, Berlin, pp 269–318. https://doi.org/10.1007/978-1-4615-4111-0_9

Macfarlane S, Macfarlane GT, Cummings JH (2006) Review article: prebiotics in the gastrointestinal tract. Aliment Pharmacol Ther 24:701–714. https://doi.org/10.1111/j.1365-2036.2006.03042.x

Macfarlane GT, Steed H, Macfarlane S (2008) Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. J Appl Microbiol 104:305–344. https://doi.org/10.1111/j.1365-2672.2007.03520.x

Manning TS, Gibson GR (2004) Prebiotics. Best Pract Res Clin Gastroenterol 18:287–298

Mishra SS, Behera PK, Kar B, Ray RC (2018) Advances in probiotics, prebiotics and nutraceuticals. In: Innovations in technologies for fermented food and beverage industries. Springer, New York, pp 121–141. https://doi.org/10.1007/978-3-319-74820-7_7

Montonen J, Knekt P, Järvinen R, Aromaa A, Reunanen A (2003) Whole-grain and fiber intake and the incidence of type 2 diabetes. Am J Clin Nutr 77:622–629. https://doi.org/10.1093/ajcn/77.3.622

Moreau NM, Martin LJ, Toquet CS, Laboisse CL, Nguyen PG, Siliart BS, Dumon HJ, Champ MMJ (2003) Restoration of the integrity of rat caeco-colonic mucosa by resistant starch, but not by fructo-oligosaccharides, in dextran sulfate sodium-induced experimental colitis. Br J Nutr 90: 75–85. https://doi.org/10.1079/BJN2003867

Navarro F, Hanauer SB (2003) Treatment of inflammatory bowel disease: safety and tolerability issues. Am J Gastroenterol 98:S18–S23. https://doi.org/10.1016/j.amjgastroenterol.2003.11.001

Nitzan O, Elias M, Peretz A, Saliba W (2016) Role of antibiotics for treatment of inflammatory bowel disease. World J Gastroenterol 22:1078–1087. https://doi.org/10.3748/wjg.v22.i3.1078

O'Hara AM, Shanahan F (2006) The gut flora as a forgotten organ. EMBO Rep 7:688–693. https://doi.org/10.1038/sj.embor.7400731

Olano A, Corzo N (2009) Lactulose as a food ingredient. J Sci Food Agric 89:1987–1990. https://doi.org/10.1002/jsfa.3694

Pasqualetti V, Altomare A, Guarino MPL, Locato V, Cocca S, Cimini S, Palma R, Alloni R, De Gara L, Cicala M (2014) Antioxidant activity of inulin and its role in the prevention of human colonic muscle cell impairment induced by lipopolysaccharide mucosal exposure. PLoS One 9: e98031. https://doi.org/10.1371/journal.pone.0098031

Pool-Zobel BL (2005) Inulin-type fructans and reduction in colon cancer risk: review of experimental and human data. Br J Nutr 93:S73–S90. https://doi.org/10.1079/BJN20041349

Pranami D, Sharma R, Pathak H (2017) Lactulose: a prebiotic, laxative and detoxifying agent. Drugs Ther Perspect 33:228–233. https://doi.org/10.1007/s40267-017-0384-z

Roberfroid M (2007) Prebiotics: the concept revisited. J Nutr 137:830S–837S. https://doi.org/10.1093/jn/137.3.830S

Russell RI (2001) Non-steroidal anti-inflammatory drugs and gastrointestinal damage—problems and solutions. Postgrad Med J 77:82. https://doi.org/10.1136/pmj.77.904.82

Ruszkowski J, Witkowski JM (2019) Lactulose: patient-and dose-dependent prebiotic properties in humans. Anaerobe 59:100–106. https://doi.org/10.1016/j.anaerobe.2019.06.002

Samanta AK, Jayapal N, Jayaram C, Roy S, Kolte AP, Senani S, Sridhar M (2015) Xylooligosaccharides as prebiotics from agricultural by-products: production and applications. Bioact Carbohydr Diet Fibre 5:62–71. https://doi.org/10.1016/j.bcdf.2014.12.003

Sarna JR, Furtado S, Brownell AKW (2013) Neurologic complications of metronidazole. Can J Neurol Sci 40:768–776. https://doi.org/10.1017/s0317167100015870

Scholz-Ahrens KE, Schaafsma G, van den Heuvel EGHM, Schrezenmeir J (2001) Effects of prebiotics on mineral metabolism. Am J Clin Nutr 73:459s–464s. https://doi.org/10.1093/ajcn/73.2.459s

Scholz-Ahrens KE, Ade P, Marten B, Weber P, Timm W, Aҫil Y, Glüer C-C, Schrezenmeir J (2007) Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. J Nutr 137:838S–846S. https://doi.org/10.1093/jn/137.3.838S

Shanahan F (2002) The host–microbe interface within the gut. Best Pract Res Clin Gastroenterol 16: 915–931. https://doi.org/10.1053/bega.2002.0342

Shoaf-Sweeney KD, Hutkins RW (2008) Adherence, anti-adherence, and oligosaccharides: preventing pathogens from sticking to the host. Adv Food Nutr Res 55:101–161. https://doi.org/10.1016/S1043-4526(08)00402-6

Slavin J (2013) Fiber and prebiotics: mechanisms and health benefits. Nutrients 5:1417–1435. https://doi.org/10.3390/nu5041417

Su CG, Wen X, Bailey ST, Jiang W, Rangwala SM, Keilbaugh SA, Flanigan A, Murthy S, Lazar MA, Wu GD (1999) A novel therapy for colitis utilizing PPAR-gamma ligands to inhibit the epithelial inflammatory response. J Clin Invest 104:383–389. https://doi.org/10.1172/JCI7145

Sun J, Chang EB (2014) Exploring gut microbes in human health and disease: pushing the envelope. Genes Dis 1:132–139. https://doi.org/10.1016/j.gendis.2014.08.001

Tanaka R, Takayama H, Morotomi M, Kuroshima T, Ueyama S, Matsumoto K, Kuroda A, Mutai M (1983) Effects of administration of TOS and Bifidobacterium breve 4006 on the human fecal flora. Bifidobact microflora 2:17–24. https://doi.org/10.12938/bifidus1982.2.1_17

Tuohy KM, Rouzaud GCM, Bruck WM, Gibson GR (2005) Modulation of the human gut microflora towards improved health using prebiotics-assessment of efficacy. Curr Pharm Des 11:75–90. https://doi.org/10.2174/1381612053382331

Tzortzis G, Vulevic J (2009) Galacto-oligosaccharide prebiotics BT - prebiotics and probiotics science and technology. In: Charalampopoulos D, Rastall RA (eds) . Springer, New York, pp 207–244

Tzounis X, Rodriguez-Mateos A, Vulevic J, Gibson GR, Kwik-Uribe C, Spencer JPE (2011) Prebiotic evaluation of cocoa-derived flavanols in healthy humans by using a randomized, controlled, double-blind, crossover intervention study. Am J Clin Nutr 93:62–72. https://doi.org/10.3945/ajcn.110.000075

Vanhoof K, De Schrijver R (1995) Effect of unprocessed and baked inulin on lipid metabolism in normo-and hypercholesterolemic rats. Nutr Res 15:1637–1646. https://doi.org/10.1016/0271-5317(95)02034-3

Vieira A, Teixeira M, Martins F (2013) The role of probiotics and prebiotics in inducing gut immunity. Front Immunol 4:445. https://doi.org/10.3389/fimmu.2013.00445

Vigsnæs LK, Holck J, Meyer AS, Licht TR (2011) In vitro fermentation of sugar beet arabino-oligosaccharides by fecal microbiota obtained from patients with ulcerative colitis to selectively stimulate the growth of Bifidobacterium spp. and Lactobacillus spp. Appl Environ Microbiol 77: 8336–8344. https://doi.org/10.1128/AEM.05895-11

Vulevic J, Juric A, Walton GE, Claus SP, Tzortzis G, Toward RE, Gibson GR (2015) Influence of galacto-oligosaccharide mixture (B-GOS) on gut microbiota, immune parameters and metabonomics in elderly persons. Br J Nutr 114:586–595. https://doi.org/10.1017/S0007114515001889

Wang S, Li C, Copeland L, Niu Q, Wang S (2015) Starch retrogradation: a comprehensive review. Compr Rev Food Sci Food Saf 14:568–585. https://doi.org/10.1111/1541-4337.12143

Watzl B, Girrbach S, Roller M (2005) Inulin, oligofructose and immunomodulation. Br J Nutr 93: S49–S55. https://doi.org/10.1079/BJN20041357

Weng M, Walker WA (2006) Bacterial colonization, probiotics, and clinical disease. J Pediatr 149: S107–S114. https://doi.org/10.1016/j.jpeds.2006.06.061

Whelan K (2013) Mechanisms and effectiveness of prebiotics in modifying the gastrointestinal microbiota for the management of digestive disorders. Proc Nutr Soc 72:288–298. https://doi.org/10.1017/S0029665113001262

Whelton SP, Hyre AD, Pedersen B, Yi Y, Whelton PK, He J (2005) Effect of dietary fiber intake on blood pressure: a meta-analysis of randomized, controlled clinical trials. J Hypertens 23:475. https://doi.org/10.1097/01.hjh.0000160199.51158.cf

Wijnands MVW, Schoterman HC, Bruijntjes JP, Hollanders VMH, Woutersen RA (2001) Effect of dietary galacto-oligosaccharides on azoxymethane-induced aberrant crypt foci and colorectal cancer in Fischer 344 rats. Carcinogenesis 22:127–132. https://doi.org/10.1093/carcin/22.1.127

Williams D, Verghese M, Walker LT, Boateng J, Shackelford L, Chawan CB (2007) Flax seed oil and flax seed meal reduce the formation of aberrant crypt foci (ACF) in azoxymethane-induced colon cancer in Fisher 344 male rats. Food Chem Toxicol 45:153–159. https://doi.org/10.1016/j.fct.2006.08.014

Wilson B, Whelan K (2017) Prebiotic inulin-type fructans and galacto-oligosaccharides: definition, specificity, function, and application in gastrointestinal disorders. J Gastroenterol Hepatol 32: 64–68. https://doi.org/10.1111/jgh.13700

Wolfe MM, Lichtenstein DR, Singh G (1999) Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. N Engl J Med 340:1888–1899. https://doi.org/10.1056/NEJM199906173402407

Wong JMW, De Souza R, Kendall CWC, Emam A, Jenkins DJA (2006) Colonic health: fermentation and short chain fatty acids. J Clin Gastroenterol 40:235–243

Zaman SA, Sarbini SR (2016) The potential of resistant starch as a prebiotic. Crit Rev Biotechnol 36:578–584. https://doi.org/10.3109/07388551.2014.993590

Zhang G, Hamaker BR (2009) Slowly digestible starch: concept, mechanism, and proposed extended glycemic index. Crit Rev Food Sci Nutr 49:852–867. https://doi.org/10.1080/10408390903372466

Prebiotics and Probiotics as Functional Foods: Prospect and Promises in Metabolic Diseases

9

Tanuka Das, Tandrani Das, and Goutam Ghosh

Abstract

A huge plethora of dynamic microbial communities present in and on the human body have a significant impact both on local (as for the gut microbiota on energy metabolism and obesity) and on distant scales (as the association of the periodontal disease with coronary heart disease). This microbiota contributes significantly to the host biology including digestion, metabolism, extraction of nutrients, synthesis of vitamins, and prevention against pathogenic colonization and also the modulation of the immune system in the host body. The composition and diversity of these microbiota have a strong association with gastrointestinal tract disorders as well as many metabolic disorders including type 2 diabetes mellitus (T2DM), cardiovascular diseases (CVDs), obesity, hypercholesterolemia, hypocholesterolemia, etc. which present an increasing public health concern and can hamper host's quality of life. Probiotics are living organisms that exert their beneficial effect on host health, whereas prebiotics are nondigestible food ingredients that benefit the host by selectively stimulating the growth or activity of one or a limited number of microbes in the host. The present state of knowledge indicates that careful manipulation of the gut microbiota could be a promising approach for the prevention and management of metabolic diseases. Manipulation of gut microbiota through the administration of prebiotics and probiotics may assist in weight loss, reducing plasma blood glucose levels and also serum lipid levels, decreasing CVD and T2DM. We analyze currently available data to ascertain further benefits and limitations of probiotics and prebiotics in the treatment of metabolic diseases. The focus of this review is to examine the role of the microbiome in most morbid human metabolic diseases

T. Das · T. Das · G. Ghosh (✉)
Department of Zoology, Midnapore College (Autonomous), Midnapore, West Bengal, India
e-mail: goutam.ghosh@midnaporecollege.ac.in

K. K. Behera et al. (eds.), *Prebiotics, Probiotics and Nutraceuticals*,
https://doi.org/10.1007/978-981-16-8990-1_9

and to highlight the current challenges and discussion areas of their prevention and management with applications of prebiotics and probiotics as functional foods.

Keywords

Metabolic disorders · Gut microbiota · T2DM · CVD · Functional foods

9.1 Introduction

Metabolic disorders, like type 2 diabetes mellitus (T2DM), cardiovascular diseases (CVDs), obesity, hypercholesterolemia, etc., in modern civilizations have created unprecedented health and socioeconomic problem and increased human mortality throughout the world (McCracken and Phillips 2017; Institute of Health Metrics and Evaluation 2019). Significant changes in dietary macronutrient consumption and altered lifestyle in the last few decades have significantly shot up the occurrence of metabolic disorders (Yoo and Kim 2016). Currently, scientists have found that metabolic disorders might result from an alteration in gut microbiota composition (Nagatomo and Tang 2015; Yoo and Kim 2016). Different microbiota in the human intestine perform many vital functions, viz., nutrient absorption, mucosal barrier protection, xenobiotic metabolism, intestine maturation, etc. (Nagpal et al. 2012). Our daily food consumption alters the composition of microbiota in the gut, which has been found to be critically linked to the development of metabolic disorders (DiBaise et al. 2008).

Microorganisms form successful ecological communities of commensal and symbiotic and establish pathogenic interactions found in and on all multicellular organisms studied to date including the humans (Peterson et al. 2009; Lloyd-Price et al. 2016). However, it is estimated that they quite often outnumber the host cells by an order of magnitude (Honey 2008; Delzenne et al. 2011). Though they are mostly concentrated in the gut, they can be found in every organ, viz., skin, oral cavity, respiratory and urinogenital tracts, etc. Much of this untrammeled microbial diversity largely remains unexplained; recent discoveries indicate the implication of diet, environment, host genetics, and early-life microbial exposure (Huttenhower et al. 2012). These microbiota are continuously evolving with the host as depicted in the "hologenome theory of evolution" (Rosenberg and Zilber-Rosenberg 2013) which advocates that "an object of natural selection is not the individual organism, but the organism together with its associated organisms, including its microbial communities" (Bordenstein and Theis 2015).

It has been suggested that the human microbiome consists of an almost invariable "core microbiome" that is common to all individuals and a "variable microbiome" that is unique to individuals (Zaura et al. 2014). The "variable microbiome" composition depends on a number of determinants—sex, age, mode of birth (i.e., natural vs. cesarean), host genetic and physiological variations, and of course, the lifestyle of the host. More than 700 oral bacterial species (Kilian et al. 2016; Benn et al. 2018) of "core microbiome" primarily consist of the *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Fusobacteria* (Zaura et al. 2009; He and Shi 2017). However, it should be noted, as de la Visitación et al. (2019) remarked, that a "model healthy human microbiome" is difficult to define and encompass considering huge individual variations and plasticity.

In recent years, the human intestinal microbiota has gained increasing interest in its impact on human health, such as its comprehensive physiological and pathological functions. The human gut is the most popular niche for the microbiota, providing a refuge of 10^{10}–10^{12} live microorganisms per gram in the colon (Collins and Reid 2016) belonging to at least 1000 different species in our gastrointestinal (GI) tract. This intestinal microbiota composition is the culmination of a duplex interaction between the host and its microbial plethora. It has been reported that some endogenous factors, such as mucin secretions (Rokhsefat et al. 2016); immune factors, such as secretory IgA (Kawamoto et al. 2012); etc. and the genetic blueprint of the host (Lloyd-Price et al. 2016) affect the composition of the intestinal microbiota. Apart from these endogenous modulations on the host's behalf, the composition and stability of the intestinal microbiota could be determined and patterned by nutrition or other factors, such as probiotics, prebiotics, antibiotics, drugs, and, even, host pathophysiology. Microbiota have been documented to be crucial for immunologic, hormonal, and metabolic homeostasis of their host.

Recently, various naturally occurring compounds have been tested to determine their function as prebiotics. Fructooligosaccharides (FOS) from fruits and vegetables, galactooligosaccharides (GOS) from milk, and *trans*-galactooligosaccharides (TOS) from dairy products, beans, and certain root vegetables are the most common prebiotics (Davani-Davari et al. 2019). Fermentation of prebiotics by gut microbiota yields short-chain fatty acids (SCFAs), viz., lactic acid, butyric acid, and propionic acid, representing 90–95% of the SCFA present in the colon (Al-Lahham et al. 2010; Wong et al. 2012). These are fermentation products from food components that are unabsorbed/undigested in the small intestine. These products can have dual effects. Firstly, these directly provide systemic health benefits to the host and, at the same time, bring about augmentation to microbiota composition and reduce the risk of gut-related diseases as well as some metabolic syndromes, such as obesity, diabetes, CVDs, chronic kidney disease, and systemic inflammation (Wang et al. 2019).

Current evidence suggests that manipulation of the gut microbiota by probiotics and prebiotics could be a promising approach for the prevention and management of metabolic disorders (Hur and Lee 2015; Boulangé et al. 2016; Marchesi et al. 2016). Consequently, the positive health benefits imputed to probiotics and prebiotics have drawn commercial interest in exploiting different applications leading to the rapid growth and expansion of the "functional food" market sector (Pintado et al. 2014).

In this chapter, we summarize the current knowledge about the application of probiotics and prebiotics in the treatment and management of metabolic diseases and how could they do qualify as prospective functional foods in the forthcoming era.

9.2 Definition and History of Probiotics and Prebiotics

"Probiotic" derives from a Greek root meaning for the "life." It was for the first time appeared in the literature in the 1960s, though the beneficial effects of certain microorganisms (particularly lactic acid bacteria and bifidobacteria) have been studied since the nineteenth century. As depicted by the international bodies and endorsed by contemporaries, probiotics is defined as:

> The live microorganisms that, when administered in adequate amounts, confer a health benefit on the host while enhancing the properties of intestinal flora. (FAO/WHO 2002)

The use of probiotics by human societies can be dated back to Roman civilization when Pliny the Elder (76 BC) recommended fermented milk to treat diarrhea (Cruchet et al. 2015). Leaping forward to modern times, early in the last century, Tissier observed that significant colonization of bifidobacteria in the gut protected against the pediatric diarrhea, and Elie Metchnikoff, Nobel laureate of Physiology or Medicine, illustrated the health benefits of *Lactobacillus bulgaricus* in fermented yogurt by restricting the growth of toxin-producing intestinal bacteria (Czerucka et al. 2007; Cruchet et al. 2015).

However, the term "probiotic" is sometimes inappropriately used as a synonym to indicate beneficial members of commensal microbiota. So, it could be meaningfully remarked that though many of human commensal microbiota are often the sources of probiotics, until such strains are isolated and then adequately characterized for content, consistency, and pro-health effects, they should not be qualified as probiotics (Sanders 2008).

An alternative approach for microflora management through diet is the use of prebiotics, which can alter the indigenous gut microbiota composition to yield positive health effects. These were first developed in order to positively influence the gut microbes and to address the survivability issues that commonly occur with probiotics, primarily due to the harsh stomach environment. The term prebiotic was first coined in the mid-1990s by Gibson and Roberfroid (1995), and there are variable dialogues and controversies among the scientific communities in the context of defining prebiotic; still the comprehensive and fairly consensus one states prebiotics as

> A non-digestible compound that, through its metabolization by microorganisms in the gut, modulates the composition and/or activity of the gut microbiota, thus, conferring a beneficial physiological effect on the host. (Bindels et al. 2015)

The term "functional food" first surfaced in Japan in the 1980s when the Japanese government focused their research endeavors on studying functional food or foods for specific health uses (FOSHU) and defined as "food products fortified with special constituents that possess advantageous physiological effects" (Hardy 2000). Ever since, the definition of functional foods is continuously evolving and eclipsing the other (Martirosyan and Singh 2015). However, a workable definition could be

“Natural or processed foods that contain biologically active compounds; which, in defined, effective, and non-toxic amounts, provide a clinically proven and documented health benefit utilizing specific biomarkers for the prevention, management, or treatment of chronic disease or its symptoms” as given by the FCC (Gur et al. 2018).

Probiotics and prebiotics can protect the human health from different metabolic disorders involved in several ways—by nurturing beneficial microflora in the gut (Kasubuchi et al. 2015), by improving gut immunity, by competing with the pathogen to prevent them from receptor binding (Tolhurst et al. 2012), by secreting extracellular proteins that induced mucosa-bacteria interaction (Sánchez et al. 2010), etc.

Among many others, CVDs, obesity, and T2DM are the most prevalent metabolic disorders contributing to significant mortality and invariably inviting a compromise to a quality life in modern human societies. There is a strong connection between the gut microbiome and these diseases as established from many recent studies (Wells et al. 2010; Tsai et al. 2019). The consumption of various commonly available fermented products enriches the beneficial microbial colony in the gut and lowers the chance of CVDs (Howell 1988; Ooi and Liong 2010; Tsai et al. 2019). Obesity may cause a change in the composition of the gut microbiota by creating light inflammation. Intake of probiotics may lower the chance of obesity by lowering lipid and glucose concentration and by the elevation of insulin level (Poirier et al. 2006). Fermented milk is very effective for the patient with hypercholesterolemia (Lewis and Burmeister 2005). Microorganisms present in probiotics compete with the harmful pathogens and prevent them from binding with the receptor, thus lowering many GI diseases in the host (Ahn and Stiles 1990; Lebeer et al. 2010). Probiotic yogurt is very much effective in the treatment of T2DM (Ejtahed et al. 2011). Administration of probiotics can promote the secretion of many hormones like insulin and glucagon-like peptide 1 (GLP-1). It also increases the level of SCFA and butyrate and can manipulate the “microbiota-SCFA-hormone axis” as found during the treatment of obesity and diabetes in different mouse models (Yadav et al. 2013). Due to low adverse effects and high effectiveness in several metabolic disorders, probiotic consumption is increasing day by day. Still, broad-scale clinical studies are needed for the fruitful implementation of probiotics in our daily life (Yoo and Kim 2016).

Some authors have introduced another related term in this context—the synbiotic (Gibson and Roberfroid 1995) that is essentially a combination of probiotics and prebiotics. It has been observed when probiotics are combined with prebiotics, the latter can improve the survival of the bacteria through the harshest course (very low pH), i.e., the upper part of the GI tract, thus enhancing their effects in the large intestine by the so-called “prebiotic effects.” Moreover, probiotics and prebiotics may exert additive or even synergistic effects (Roberfroid 2000).

9.3 Prebiotics, Probiotics, and Host Physiology

Prebiotics are defined as nondigestible food resources which are beneficial for the host by stimulating the growth and activity of many bacteria in the colon (Chen and Walker 2005). The role of probiotics is to produce and secrete antimicrobial substances that stimulate the host's immune responses and to fight the pathogen assault. Eventually prebiotic has been described as partially fermented ingredients that promote changes in the composition and activity of microflora beneficial for the health of the host (Roberfroid 2007). As suggested by Gibson et al. (2004), effective prebiotics should possess three important properties: (1) resistant to low gastric pH, digested by mammalian enzymes, and readily and effectively absorbed in the GI tract, (2) fermented by intestinal microflora, and (3) stimulate and positively regulate the expansion and activity of intestinal bacteria related to health. Fructooligosaccharides, galactooligosaccharides, lactulose, and nondigestible carbohydrates (inulin, resistant starches, cellulose, hemicellulose, pectin, and gums) are prebiotics that meet the above three properties. Most prebiotics like fructooligosaccharides and inulin are hydrolyzed by *Bifidobacterium* that promote the homeostasis of intestinal cells and inhibit pathogenic bacterial growth (Gargari et al. 2013). Acetic acid, propionic acid, and butyric acid are short-chain fatty acids (SCFAs) that are important end products of carbohydrate metabolism. SCFAs have been reported to decrease the chance of GI disorders, cardiovascular diseases, and cancers by inducing apoptosis (Slavin 2013; McNabney and Henagan 2017). Moreover, prebiotics could induce the immune system, produce vitamin B, inhibit pathogen growth, and decrease blood ammonia. They also aid in cellular differentiation and regulate cell-cycle checkpoints and apoptosis by inhibiting the enzyme histone deacetylase (Gibson and Roberfroid 1995). Furthermore, SCFAs can reduce glucagon levels in a dose-dependent manner, increase glucose tolerance, and activate glucagon-like peptide1 (GLP-1), which can induce the insulin production and reinstate insulin sensitivity (Everard et al. 2011). In this way, prebiotics exert an important role in the improvement of intestinal metabolism. The term probiotics has also been used to describe the secretions of one microorganism which induce the growth of another (Chen and Walker 2005). They are beneficial for the health of the host because they enhance metabolic activities or protect against intestinal infection, food allergies, and colon cancer (Chen and Walker 2005). Probiotics also have a major function as a potential dietary supplement not only in the intestinal tract but also in systemic metabolic disorders, such as type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CVD) (Nagpal et al. 2012). Patients with T2DM have a lower number of bacteria that produce butyrate in comparison with healthy people. There is an association between T2DM and compositional changes in the intestinal microflora, viz., phyla *Firmicutes* and *Bifidobacterium* are found in a lower amount in T2DM patients than in nondiabetic individuals (Wu et al. 2010). Probiotics and prebiotics may improve lipid profiles, including the reduction of LDL cholesterol, plasma total cholesterol, and triglycerides or the induction of high-density lipoprotein (HDL cholesterol) - important biomarkers associated with CVDs (Ejtahed et al. 2011).

9.3.1 Protective Properties of Prebiotics and Probiotics

Prebiotics and probiotics protect humans from different metabolic disorders by the following properties:

9.3.1.1 Host Defense by the Formation of the Bacterial Colony

After birth, intestinal mucosal host defense is established by initial bacterial colonization, primarily *Lactobacilli* and *Bifidobacterium*. It is initiated with the feeding of breast milk containing nondigestible oligosaccharides (Prebiotic factors) (Balmer and Wharton 1989). Pathogenic bacteria cause infection due to insufficient protective bacterial colonies in the human intestine. Probiotics inhibit the proliferation of pathogenic organisms by secreting antibiotic substances that prevent pathogenic bacterial adherence to the intestinal surface (Walker 2000).

9.3.1.2 Induction of Gut Immunity

The intraepithelial T-lymphocytes and some B-lymphocytes present in the Peyer's patches, lymphoid follicles, and lamina propria in gut-associated tissue can protect the intestinal mucosa by actively destroying pathogenic organisms. Probiotics promote these host defense mechanisms by enhancing humoral immune responses, thereby stringing the intestinal immunologic protection (Phieler et al. 2013). Furthermore, probiotics can downregulate hypersensitivity reactions by augmenting nonspecific immune response in the host toward microbial pathogens and able to mount some resistance against potentially harmful allergens (Meydani and Ha 2000).

9.4 Food as a Vehicle of Probiotics

Nowadays, the demand for functional foods has increased globally, particularly in developed countries (Siró et al. 2008; Granato et al. 2010). These foods include probiotics and prebiotics as biologically active components, demanding to be producing metabolic and physiological health benefits. It is known that the ingestion of some microbial strains provokes health benefits (Nagpal et al. 2012). So, high numbers of viable bacteria are recommended to assure probiotic food efficiency. The amount of intake and form of the probiotic food should be adequate for dietary purposes. Probiotic bacteria are currently used to be incorporated into food products. But some challenges must be faced when it is included in food matrices with more aggressive environments like high salt, acid, or oxygen concentrations or low temperatures (Mattila-Sandholm et al. 2002). Furthermore, the beneficial effects of probiotic bacteria rely on its viability, requiring those food matrices as carriers to assure their viability at high levels for the entire shelf life to guarantee their passage throughout the gastrointestinal tract and colonization of gut mucosa for beneficial effects (Mattila-Sandholm et al. 2002). Dairy products, such as fermented milk, cheeses, etc., are mainly able to carry and deliver probiotic bacteria. Milk and dairy products contain some nutritional characteristics that allow the growth and survival of probiotic bacteria. Some dairy products, especially fermented milk and cheeses, contain appropriate pH and buffer capacity, dense matrix, and fat content that help in additional protection to these microorganisms in their passage through the GI tract (Behera et al. 2018; Terpou et al. 2019).

9.5 Functional Foods and Metabolic Diseases

Functional foods play a prime role in our daily health and well-being (McClements 2019). Functional foods are being used in ameliorating such health problems like obesity, diabetes, cardiovascular diseases (CVDs), cancer, osteoporosis, allergies, and dental problems (Valls et al. 2013; Gul et al. 2016). Some microorganisms (probiotics) play an important role in host health with their immunological and physiological functions (Cencic and Chingwaru 2010). Probiotics and prebiotics are the two categories of lactic fermentation products in functional foods. Probiotics, such as *Lactobacilli* and *Bifidobacterium*, are mainly involved in improving the bacterial balance of our intestine. Nondigestible food ingredients, such as oligosaccharides and dietary fibers, are prebiotics that influence the host by refreshing growth or activities of probiotics in the colon that improve the host health. In this way, functional foods help to prevent such diseases like diabetes, CVD, cholesterolemia, cancer, etc. (Mitsuoka 2014). Functional foods that are found to be important in cardiovascular patients are dietary fiber, long-chain ω-3 fatty acids, phytochemicals, and vegetable proteins such as soy proteins (Alissa and Ferns 2012). Type 2 diabetes is also a convoluted disorder with both short- and long-term unacceptable complications. Functional foods and their bioactive compounds may be used as a supportive treatment for T2DM (Alkhatib et al. 2017). Functional foods could prevent the development of long-term diabetes complications including cardiovascular disease, nephropathy, neuropathy, and retinopathy by increasing insulin resistance and reducing inflammatory processes and oxidative stress (Mirmiran 2014). Therefore, a healthy diet based on some functional foods (fruits, vegetables, whole-grain foods, low saturated fat products, low glycemic index starchy foods, etc.) can serve as a novel dietary approach for the management of type 2 diabetes (Riccardi et al. 2005).

9.6 Protective Role of Probiotics and Prebiotics in Metabolic Diseases

Probiotics have been found to play a stellar role in metabolic diseases, such as cardiovascular disease, diabetes, obesity, etc. (Table 9.1). A few of the most common metabolic diseases are discussed in the following sections.

Table 9.1 Some of the probiotics and prebiotics used to treat metabolic disorders with their respective functions

Name of probiotics and prebiotics	Subject(s)	Functions	References
1. Probiotics			
(a) Fermented yogurt with:.			
Lactobacillus bulgaricus, *L. acidophilus*, *Bifidobacterium lactis*	Human	1. Improve total cholesterol and LDL cholesterol concentrations in T2DM people	Ejtahed et al. (2011), Cruchet et al. (2015)
		2. Improve fasting blood glucose and antioxidant status in T2DM patients	Ejtahed et al. (2012)
		3. Improve dyslipidemia in T2DM patients	Mohamadshahi et al. (2014)
(b)Fermented milk with:.			
• *Bifidobacterium lactis*	Human	1. Prevention of acute infectious diarrhea	Cruchet et al. (2015)
		2. Reduce risk for antibiotic-associated diarrhea and reduce duration of acute infectious diarrhea with about 24 h	Vandenplas et al. (2013)
• *Lactobacillus acidophilus*	Human	Reduce serum lipids in hypercholesterolemic patients	Lewis and Burmeister (2005)
• *L. gasseri*	Rat	Lowering the effect of abdominal adiposity, body weight etc.	Kadooka et al. (2010)
(c) Fermented soy milk with *Lactobacillus plantarum* A7	Human	Improve the kidney function with reduced urine albumin and serum creatinine level, improve GFR	Abbasi et al. (2017, 2018)
(d) Cheese with *Lactobacillus fermentum*	Human	1. High antimicrobial activity associated with organic acids and hydrogen peroxide production	Annuk et al. (2003)
		2. Antioxidative property present and decrease the risk of reactive oxygen species (ROS) accumulation in host and used as oxidative stress-reducing probiotic food supplement	Kullisaar et al. (2002)
(e) Food with *Lactobacillus johnsonii*	Human	Beneficial for human fecal microbiota	Cruchet et al. (2015)

(continued)

Table 9.1 (continued)

Name of probiotics and prebiotics	Subject(s)	Functions	References
(f) Indian yogurt with *Lactobacillus acidophilus*, *L. casei*	Mice	1. Inhibit insulin depletion and preserving diabetic dyslipidemia 2. Inhibit lipid peroxidation 3. Reduction of insulin and elevation of blood glucose levels	Yadav et al. (2007)
(g) Food supplements with strains of *Lactobacillus*, *Bifidobacterium*, and *Streptococcus*	Human	Decrease in fasting plasma glucose and increase in serum HDL-C	Razmpoosh et al. (2019)
(h) *Bifidobacterium animalis* subsp. *lactis* F1-3-2	Mice	Improve the lipid metabolism and reduce atherosclerosis caused by TMAO	Liang et al. (2020)
(i) *Lactobacillus rhamnosus* (Lbl02) and *Bifidobacterium animalis* ssp. *lactis* Bfl41	High-fat high-sucrose-fed obese mice	1. Reduction in visceral adiposity 2. Improvement of insulin sensitivity	Le Barz et al. (2019)
2. Prebiotics			
(a) Prebiotic fiber with inulin food supplement	Rat	Reduced plasma glucose levels	Reimer and Russell (2008)
	Human	1. Promote homeostasis in intestinal cells 2. Inhibit pathogenic bacterial growth 3. Improvement of glycemic and antioxidant indices in women with T2DM	Gargari et al. (2013)
(b) Food with oligofructose supplement	Human	1. Reduce body weight 2. Suppressed ghrelin and enhanced peptide YY (satiety hormone)	Parnell and Reimer (2009)
(c) Food with dietary fiber (fructooligosaccharides, inulin)	Human	1. Less risk of CVD and play a role in gut health 2. It stimulates *Bifidobacterium*, a beneficial genus that is effective in CVD and T2DM patients	Slavin (2013)
		1. Improve hyperglycemia and promote weight reduction in treating T2DM 2. Influence host by refreshing growth or activities of probiotics in the colon	Mitsuoka (2014)

(continued)

Table 9.1 (continued)

Name of probiotics and prebiotics	Subject(s)	Functions	References
(d) Foods with glycans (glycosaminoglycans) and polysaccharides including dietary plants (starch, hemicelluloses, pectin)	Human	1. Act as agents linked to CVD, cancer, and obesity and maintain health 2. Promote proliferation of beneficial microbes in the human gut	Koropatkin et al. (2012), Gargano and Hughes (2014)
(e) Foods containing oligodextran and lactose	Human	1. Weight loss; reduce plasma glucose levels and serum lipid levels 2. Reduce risk of CVD, T2DM 3. Regulate insulin secretion fat accumulation, energy homeostasis, plasma cholesterol levels	Gibson et al. (2004), He and Shi (2017)
(f) SCFA containing *Clostridium butyricum*	Mice	1. Increase insulin resistance	Slavin (2013)
	Human	2. Improve glucose homeostasis, leptin sensitivity, and target enteroendocrine cell activity	Everard et al. (2011)
	Human	3. Decrease the chance of GI disorders, CVDs and, cancer by inducing apoptosis	Slavin (2013), McNabney and Henagan (2017)
(g) Food with long-chain fatty acid, phytochemicals, and soy proteins	Human	1. Important in chance of lowering CVD	Alissa and Ferns (2012)
	Human	2. Decrease in body weight and BMI 3. Reduction in atherogenic lipid markers (TC, LDL-C, non-HDL-C, and apoB)	Ruscica et al. (2018)
(h) Foods containing dietary carbohydrates with fructooligosaccharides, galactooligosaccharides, lactulose	Human	1. Resistance to gastric acidity hydrolysis by mammalian enzymes and GI absorption fermentation by intestinal microflora 2. Stimulation of growth and activity of intestinal bacteria associated with health	Gibson et al. (2004)
3. Synbiotics			
Synbiotic mixture in pasta (prebiotic barley β-glucans and probiotic *Bacillus coagulans*)	Human	Improved plasma hs-CRP, plasma LDL/HDL cholesterol ratio, in sedentary overweight and obese adults	Angelino et al. (2019)
Lactobacillus rhamnosus and inulin	Human	Reduction in body weight loss and reduction in leptin level	Sanchez et al. (2014)
Several strains of *Lactobacillus*, *Bifidobacterium*, and *Enterococcus faecium*, fructooligosaccharides	Human	Decrease in TC, LDL-C and total oxidative stress parameters in serum levels	Ipar et al. (2015)

9.6.1 Prebiotics and Probiotics in the Management of Type 2 Diabetes (T2DM)

Nowadays, T2DM has emerged as a wide health issue for human and drastically affects the socioeconomic condition across the globe. It is a multifactorial disorder and creates several other health issues like β-cell dysfunction, hyperglycemia, adiposity, hypercholesterolemia, dyslipidemia, ectopic fat storage, and ultimately cardiovascular disease (Mengual et al. 2010). Gut microbiota has been reported to be very effective for the pathogenesis of T2DM, obesity, and related inflammatory metabolic diseases. The cause for the development of T2DM is the disturbance of normal gut microflora resulted from dietary intake of high-fat and fructose diet (Zhao and Shen 2010). Lowered number of microflora belonging to the phylum Firmicutes and Clostridia and a high number of Betaproteobacteria are linked with plasma glucose level (Larsen et al. 2010). Metagenomic studies clearly have shown the changes in gut microbiota in obesity—the principal reason behind the development of T2DM in various animal and human models (Grover et al. 2012). The microbe-gut-brain axis has been closely linked with energy homeostasis (Mayer et al. 2015; Cryan et al. 2019). Changes in microbial composition hamper the circuitry of enteroendocrine signals from the gut to the central nervous system (CNS) (Rogers et al. 2016; Arneth 2018).

In this particular disease management arena, prebiotics are proposed to be safe and cost-effective in increasing the host bacterial interactions for the treatment of obesity and diabetes (Cani et al. 2009a). Recently, probiotics are being used as natural biotherapeutics or nutraceuticals for their health-inducing ability to resist diseases including T2DM (Grover et al. 2012; Panwar et al. 2013).

The therapeutic role of probiotics was studied in different cell lines, animals, and human models. It was found that probiotics are reasonably effective for the treatment of T2DM (Panwar et al. 2013). Both in vivo and in vitro studies suggest that oxidative stress is harmful for insulin-secreting beta cells and destruction could be prevented by metabolites of *Lactobacillus* sp. (Panwar et al. 2013; Valenlia et al. 2018). When the therapeutic role was studied in mice model, it was found that application of probiotic dahi (Indian yogurt) containing *L. acidophilus* NCDC14 and *L. casei* NCDC19 significantly lowers the blood glucose level, glycosylated hemoglobin, free fatty acids, and triglycerides in fructose-induced diabetic mice (Yadav et al. 2007). Several studies have been made to find the efficacy of probiotics in the treatment of human T2DM in the last decade (Sun et al. 2020). Many promising pieces of evidence have now been accumulated to establish the role of probiotics in the treatment of human T2DM. It was found that probiotic yogurt consumption lowers total serum cholesterol (Ejtahed et al. 2011; Pei et al. 2017). There was a significant reduction in glucose level following the treatment of diabetic patients with probiotics. Following the application of probiotics on T2DM patients, a balanced intestinal microflora was observed. This was probably due to the consumption of SCFA that was produced from probiotics (Sadrzadeh-Yeganeh et al. 2010). Probiotics are also very effective in recovering β-cells damage (Yadav et al. 2008).

Metformin, a commonly prescribed drug in the treatment of type 2 diabetes, has been linked to a decrease in the abundance of butyrate-producing bacteria (Larsen et al. 2010; Zhang et al. 2013) and an increase in *Escherichia* sp. in the gut (Forslund et al. 2015) potentially indicating the adverse gastrointestinal effects of metformin (Kyriachenko et al. 2019). In such instances, where the most widely prescribed drug in treating a particular ailment invites such an unwanted ill-effect, probiotics could immerge as the best alternative possible. However, some workers have suggested that the therapeutic effects of metformin are actually mediated by specific gut microbiota (Buse et al. 2016; Brunkwall and Orho-Melander 2017).

In addition, many other experiments describe that probiotics and prebiotics may play an indispensable role in the future treatment of diabetes—they may decrease the incidence, delay the onset, and reverse the extension of the complications of T2DM (Gall et al. 2010; Huttenhower et al. 2012; Zhang et al. 2020).

9.6.2 Prebiotics and Probiotics in the Management of Cardiovascular Diseases

Globally, the prevalence of CVD is on a continuous hike for the last few decades (Institute of Health Metrics and Evaluation 2019; Zhao et al. 2019). There is a correlation between microbes and CVD that may prevent the risk of CVD. The teeth, tongue, cheek, and gingiva are colonized with microbial communities (Dewhirst et al. 2010). Though the primary function of these microbiota is to maintain homeostasis in the oral cavity as mentioned earlier, their implication is also phenomenal in distant organs. Consequently, it is not surprising that the oral cavity, adipose tissue, liver, heart, and gut have been linked as the potential sites of action for probiotics in ameliorating CVD in recent years (He and Shi 2017; Cerdó et al. 2019).

The strong association between the CVDs with other metabolic dysfunctions, viz., obesity, type 2 diabetes, and inflammation, aggravates the situation further, possibly, in synergy (DeBoer 2013; Frydrych et al. 2018). Though these metabolic dysfunctions have genetic and epigenetic regulations (Slomko et al. 2012; Martínez et al. 2014; Ling and Rönn 2019), they are also diet-dependent and are risk components for CVD. Sometimes, overnutrition can re-pattern the composition of the gut microbiota. For instance, the Firmicutes predominate the gut microbiota in people consuming more cholesterol, saturated fats, and simple carbohydrates (Wong et al. 2012). The microbes 'nurtured' on modern 'Western diet', rich in lipids and simple carbohydrates (primarily, sucrose and fructose), and lack complex fermentable fibers that grossly mismatches to the composition of our 'ancestral' microbiomes (Turnbaugh et al. 2009; Ettinger et al. 2014). The unprocessed or little processed foods, whole grains consisting of fermentable complex fibers, plant and animal glycans, fresh or raw fruits, and fermented vegetables, foods, and beverages can promote the proliferation of the "typical" beneficial microbes that coevolved in the human gut niche since thousands of years of association (Koropatkin et al. 2012; Gargano and Hughes 2014; Pérez-Díaz 2019). Epidemiological studies across different parts of the globe revealed that vegetarians and vegans have generally lower blood cholesterol (Sacks et al. 1985), low-density lipoprotein cholesterol (LDL-C), high-sensitivity C-reactive protein (hs-CRP) levels (Chen and Walker 2005), and lower risk for CVD than omnivores (Key and Davey 1996; da Rosa et al. 2008; McEvoy et al. 2012). The consumption of red meat has long been associated with an increased risk for CVDs (Micha et al. 2010), that is, rich in phosphatidylcholine, choline, and carnitine. Carnitine is finally metabolized to trimethylamine-*N*-oxide (TMAO) by gut bacteria (Bennett et al. 2013). In humans, elevated levels of TMAO concentrations are associated with atherosclerosis (Koeth et al. 2013) and an increased risk of CVD (Tang et al. 2013). On contrary, vegetarians and vegans have been found to have a higher richness of beneficial bacterial species (viz., *Bacteroides*) that consequently pose a reduced risk for various CVDs and heart failure. Vegetarian diet consists of very high portions of fermentable substrates. So these components are metabolized by gut microbes that are strong evidence for the role of the microbiota in CVD (Koeth et al. 2013; Tang et al. 2013; Ettinger et al. 2014). Various food habits on the development of obesity and CVDs are being studied since long (Panagiotakos et al. 2007; Shimazu et al. 2007; Heidemann et al. 2008). Lack of physical activity, overnutrition, obesity, hypertension, T2DM, and other lifestyles (viz., smoking) are vital risk factors for myocardial infarction and coronary heart disease, all of which culminate into a higher chance to congestive heart failure (Re 2009). Another important factor in human cardio metabolism is the SCFAs. The gut microbiota has the potential to ferment nondigestible carbohydrates, like dietary fibers. These SCFAs have many functions in our metabolism and have been implicated in colonic health (Gill et al. 2018). It is known that there is an intricate relationship between SCFA and glucose/lipid metabolism (Cani 2014). Propionate and acetate act as substrates for gluconeogenesis and lipogenesis (Canfora et al. 2015), and butyrate functions as the primary source of energy for metabolism in colonic epithelial cells (Donohoe et al. 2011) that induce insulin sensitivity (Gao et al. 2009).

9.6.2.1 The Protective and the Therapeutic Effects of Probiotics Against CVD

It is well recognized that the composition of the host microbiome is grossly altered in many metabolic disorders (Frank et al. 2011; Saltzman et al. 2018). Probiotic and prebiotic consumption alters the composition of gut microbiota, and the host can effectively harvest the beneficial effects by virtue that probiotics can outcompete harmful pathogens. Restoration of a “normal microbiome” is the only way to restore a normal physiological state. Probiotic strains have been found to even pose a barrier to the pathogen strains tweaking with the host immune system (Walker 2008). Probiotics have been found to improve the integrity of epithelial barriers that are the major defense against foreign pathogens and function of tight junctions (Reid et al. 2011) but also can interact with the factors that regulate inflammatory responses (Corthésy et al. 2007). In addition, a healthy microbiome also produces many metabolites (viz., SCFAs, bile acids, bioactive lipids, polyphenols, and aromatic amino acid-derived metabolites), enzymes (e.g., bile salt hydrolases), and multiple vitamins that help in maintaining the host's health. Further, oral flora has been found to convert the diet-derived nitrate (found in high levels in leafy vegetables) to inorganic nitrate (NO_3^-) to nitrite (NO_2^-) (Hezel and Weitzberg 2015). Consequently, nitric oxide (NO) is generated, which plays a vital role in increasing vasodilatation, improving vascular endothelial function, and decreasing blood pressure (Kapil et al. 2013; Burleigh et al. 2018). Microbial nitrate reduction mainly facilitated by the taxa *Veillonella* and *Actinomyces* (Doel et al. 2005; Hyde et al. 2014). *A. odontolyticus* has been shown to have a novel probiotic potential in that it can maximize nitrate utilization from the diet and increase NO production and can reduce the risk of CVDs (Ettinger et al. 2014). Till now, the applications of probiotics for cardiovascular diseases were restricted to metabolic and diet-associated processes. Probiotics can provide a direct, cardioprotective effect to the heart that reduces the risks of other CVDs, such as coronary artery disease, myocarditis, myocardial infarction, and heart failure. It also helps to reduce ischemic injury and improve cardiac function and postinfarction (Tang et al. 2017; Kitai and Tang 2018; Jia et al. 2019). The use of probiotic *Lactobacillus plantarum* 299 v in the ischemia/reperfusion (I/R) murine model has shown promising results (Lam et al. 2012). It was found that protection against I/R injury was mediated by reduced leptin rather than cytokine-mediated pathway. Primarily produced from adipocytes and to some extent by cardiomyocytes as part of autocrine and paracrine circuitry (Purdham et al. 2004), leptin remains elevated and shows detrimental effects in CVD patients. So, this study clearly demonstrates the potential of a probiotic in the prevention of the I/R injury to a considerable extent (Ettinger et al. 2014). The use of probiotics as a management regime for CVD after a major cardiac event may be more helpful (Go et al. 2013). Several etiologies (viz., hypertension, hypertrophies, MI, coronary artery diseases, atherosclerosis) of CVDs, alone or in combination, ultimately culminate into heart failure if remained untreated or even poorly managed. It will be a futuristic and novel approach to incorporate "nature's ways" in the management or treatment of heart failure to complement the commonly used drugs, viz., ACE inhibitors, angiotensin-2 receptor blockers (ARBs), and β-blockers. Many forms of CVDs primarily originate from hypercholesterolemia and dyslipidemia (Cífková and Krajčoviechová 2015). Particularly, a high level of LDL cholesterol is most commonly associated with the most types of CVDs. Hypercholesterolemia is strongly correlated with many CVDs; consequently, most patients are prescribed with cholesterol-lowering drugs belonging to statins. These in the long run among many other side effects—perturb liver functions (Marzoa-Rivas et al. 2005), induce myalgia (Guyton 2006), or even may lead to diabetes (Sattar et al. 2010) aggravating another comorbidity factor of CVDs itself. Practicing dietary control in such a situation could be a better alternative to manage cholesterol through the administration of probiotics or prebiotics (Yoo and Kim 2016). Several lines of evidences are available in many in vivo models including human depicting the hypocholesterolemic effects of probiotics that include *Lactobacillus plantarum* PH04 (Nguyen et al. 2007), *L. reuteri* (Jones et al. 2012), *Bifidobacterium longum* Bb-46 (Abd El-Gawad et al. 2005), and *B. bifidum* (Guardamagna et al. 2014) to mention a few among hundreds. Even combination of bacterial strains has been reported to have more profound effects on total cholesterol and liver cholesterol levels (Fukushima et al. 1999). Kim et al. (2017) also demonstrated similar effect and reduction of total cholesterol and triglycerides using three strains of *Bifidobacterium* (*B. longum*, *B. lactis*, and *B. breve*) and two strains of the species *Lactobacillus* (*L. reuteri* and *L. plantarum*). Probiotics may lead to hypocholesterolemia through the three mechanisms. Firstly, enhanced cholesterol excretion leads to lower cholesterol absorption via feces (Kumar et al. 2012). Secondly, probiotic bacterial *bsh* gene-encoded enzyme bile salt hydrolase (BSH) can deconjugate cholesterol or conversion to coprostanol (Lye et al. 2010a, b). The other mechanism is production of SCFA on selective fermentation by gut microbiome from oligosaccharides (Kim and Shin 1998). Other prebiotics, such as oligodextrans, lactose, resistant and modified starches, lactoferrin-derived peptides, xylooligosaccharides, soybean oligosaccharides, and *N*-acetyl chitooligosaccharides, have been found to exert hypercholesterolemia effects in people with T2DM and, consequently, are at high risk of developing CVD (Gibson et al. 2004; He and Shi 2017). However, this should also be noted that the efficacy of the probiotics and prebiotics in the control of serum lipids also depends on different delivery systems (Yoo and Kim 2016).

9.6.2.2 Hypercholesterolemia

Hypercholesterolemia is an important cause of atherosclerosis and CVDs. To control serum cholesterol levels, some diets are being used increasingly that reduce the cholesterol, for example, fermented milk products help to reduce cholesterol. Colonic bacteria ferment food-derived oligosaccharides that lead to increased production of SCFAs, which can suppress the serum cholesterol concentrations. Probiotics can hydrolyze conjugated bile acids that are accumulated in a large amount in the gut during cholesterol metabolism. Conjugated bile acids assist in the efficient metabolism of lipids (Vaughan and Mollet 1999). Brashears et al. (1998) reported the efficacy of probiotics in deconjugating and precipitation of bile acids. It is also known that consumption of fermented milk can control LDL cholesterol (Lewis and Burmeister 2005). Apart from these, other fermented products, viz., oat-based products (Mårtensson et al. 2005), prebiotic water-soluble dietary fibers (Theuwissen and Mensink 2008), β-glucan (Sima et al. 2018), and even synbiotic plant sterol-enriched fermented milk (Plana et al. 2008), either were found to reduce serum cholesterol or enhanced the attainment of LDL cholesterol target in hypercholesterolemic patients. Hypertension is closely related to hypercholesterolemia which is one of the major risk factors for CVDs (Egan et al. 2013).

Increased level of LDL-C has long been identified as a major risk factor for CVDs (Grundy 2008). The accumulation of LDL-C in the blood invites hypertension and hyperlipidemia and also leads to the buildup of atherosclerotic plaque in the arteries. Serum LDL-C and total cholesterol levels were reduced following probiotic consumption (Guo et al. 2011). It is hypothesized that the ingestion of probiotics can sequester cholesterol by binding to their cell surface and incorporation into cellular membranes (Kimoto et al. 2002; Lye et al. 2010b). As reviewed and remarked by Ooi and Liong (2010), among several hundreds of probiotic strains, the probiotic dosage to be effective to exhibit hypocholesterolemic effects is strain-specific. However, there is an urgent need to work out the dosage and associated mechanistic pathways that will allow the better formulation of probiotics to effectively manage hypercholesterolemia.

9.6.3 Prebiotics and Probiotics in the Management of Obesity

Obesity is one of the major global health concerns due to its increasing prevalence, particularly in "developed" countries, and its association with some other metabolic disorders (James 2008) like type 2 diabetes mellitus, atherosclerosis, CVDs, nonalcoholic fatty liver disease, etc. Obesity is the culmination of an energy "dysbalance" between energy intake and energy expenditure being regulated by multiple pathways. Gut microbiome has been found to be linked with energy metabolism of the host, by regulating nutrient absorption systems and metabolic pathways, maintaining the integrity of the gut barrier, adipogenesis, neuropeptide secretion, autocrine, as well as endocrine regulation (Rawls et al. 2006). The obesity pandemic is associated with an increase in energy availability, sedentariness, and living in a controlled ambient temperature commensurate with the socioeconomic development of recent times (McAllister et al. 2009). Genetic susceptibility (Allison et al. 1996; Riveros-McKay et al. 2019), epigenetic factors (Feinberg et al. 2011; Ling and Rönn 2019), and the state of the host immune system and nutrition have a determining role in body weight. Recently, intestinal microflora has drawn increased attention as a "metabolic link" between the outer environment and the host and found to be a possible causative factor of metabolic conditions and consequently a therapeutic target to reinstate the balance. A host can select an "optimal microbiota" as a strategy of coevolution and mutual benefit even after gross perturbation if properly inoculated with (Rawls et al. 2006). High-fat diet-induced obesity and metabolic disorders have been found to be correlated with endotoxemia, inflammation, and increased infection due to the breakdown of the immune system (Cani et al. 2009a; Delzenne et al. 2011). It has been found that adipose tissue from obese humans and rodents are infiltrated by an increased number of macrophages along with the production of various inflammatory cytokines (Weisberg et al. 2003). Chronic activation of the innate immune system, which may depend on the immunomodulatory effects exerted by dietary compounds in the gut (Zeyda and Stulnig 2007), is considered as the major risk factor for obesity and related pathophysiology (Zhi et al. 2019). The human intestinal tract carries an enormous magnitude of bacterial cells that ranges from 10^7 to 10^{12} cells/g of intestinal content at different regions (Xu et al. 2007). The collective genome (metagenome) of the gut microbiota contains, as initially estimated, at least 100 times the genes (Gill et al. 2006) compared to the host genome that attributes to human interindividual diversity. However, more recent estimates suggest that it could be at least 25 times the host genome itself (Qin et al. 2010). Diet-induced obesity in mice is linked with an increased population of *Eubacterium dolichum* (division: Firmicutes) (Turnbaugh et al. 2008) and with a lower abundance of *Bifidobacterium* (Cani et al. 2007). Ley et al. (2006) reported shifts in the relative proportions of Firmicutes and Bacteroidetes in the feces of the human obese subjects. Besides, obese human adults fed with a hypoenergic diet (either low-carbohydrate or low-fat diet) show significant increases in the fecal proportions of *Bacteroidetes* commensurating to the weight after 1 year (Ley et al. 2006). It was also found from a comparative study between lean and obese twin sets that a lower proportion of Bacteroidetes and a higher proportion of Actinobacteria are the key to the development of obesity (Turnbaugh et al. 2009). Kadooka et al. (2010) reported reduction in body weight, BMI, waist and hip circumference, and visceral and subcutaneous adiposity following application of probiotic *Lactobacillus gasseri* via fermented milk. It is known that both an energy-restricted diet and increased physical activity reshape the community structure of the gut microbiota in obese individuals, correlated with weight loss and reduction in BMI *Z*-scores (Nadal et al. 2009). Alterations in the fecal microbiota composition have been linked to pediatric obesity. It is shown following the fecal content analysis that children with normal BMI have higher proportion of *Bifidobacterium*, whereas children becoming overweight have an increased number of *Staphylococcus aureus* (Kalliomäki et al. 2008). In humans, two phyla are dominant in the gut, the Gram-negative Bacteroidetes and the Gram-positive Firmicutes (Raman et al. 2010). In obese humans, lower fecal bacterial gene richness is correlated to higher overall adiposity and dyslipidemia, impaired glucose homeostasis, and elevated low-grade inflammation (Cotillard et al. 2013). Consolidating reports are there to relate alterations in intestinal microflora composition with the metabolic disorders that are almost invariably linked to obesity, such as insulin resistance, atherosclerosis, and low-grade chronic inflammation. It is also found that high-fat diets have adverse effects on gut microbiome and alter their composition. Cani et al. (2007) showed that bacterial lipopolysaccharide (LPS)-induced endotoxemia (called metabolic endotoxemia) causes low-grade chronic inflammation and invites precocious onset of metabolic diseases, such as visceral fat deposition, glucose intolerance, and hepatic insulin resistance. Serino et al. (2013) suggested LPS as the molecular link between intestinal microflora and the chronic low-grade inflammation induced by a high-fat diet that leads to insulin resistance. In addition, increased gut permeability to bacteria and their products relates to insulin resistance and oxidative stress (Cani et al. 2008). The intestinal microflora can metabolize the dietary lipid, phosphatidylcholine to trimethylamine, which predisposes mice to atherosclerosis and inflammation (Wang et al. 2011). Intestinal microflora also reported to modulate the tissue fatty acid composition. Bioactive isomers of conjugated linoleic acid synthesized by *Lactobacilli* and *Bifidobacterium* are found to be antidiabetic, anti-atherosclerotic, and immunomodulatory in nature and possess anti-obesity property (Musso et al. 2010).

The transition from overweight to obese leads to an array of comorbid factors that are harmful to the cardiovascular state (Poirier et al. 2006). The positive energy budget leads to fat accumulation in obese making them susceptible to major risk factors for type 2 diabetes, chronic inflammation, and hyperlipidemia and, moreover, predisposing them to fatal CVDs and ultimately heart failure (Ettinger et al. 2014). Lee et al. (2014) reported a significant reduction in weight, waist circumference, and serum cholesterol after 8 weeks of treatment with multi-probiotic strain mixtures (*Streptococcus thermophilus*, *Lactobacillus plantarum*, *L. acidophilus*, *L. rhamnosus*, *Bifidobacterium lactis*, *B. longum*, and *B. breve*) in overweight subjects. In addition, some peptides are produced in the gut that reduces the low-grade systemic inflammation in the obese phenotype. Gut microbiota composition affects the synthesis of these peptides. The gut microbiota regulates one such protein, i.e., serum amyloid A3 protein (SAA3), that can be expressed in adipose tissue (Cani et al. 2012). So, it is suggested that the gut microbiota control the biological systems that regulate the bioavailability of nutrients, energy storage, adipocyte gain, and inflammatory reactions in the host, each of which is correlated with the obese phenotype (Bindels et al. 2013). Significantly, the number of *Bifidobacterium* is inversely correlated with fat mass, glucose intolerance, and LPS level (Delzenne et al. 2007). In human, prebiotics such as inulin-type fructans has also been found to be effective to control metabolic disorder-related biomarkers (Reimer and Russell 2008). Similarly, Parnell and Reimer (2009) reported that the application of inulin-type fructans reduced body weight and caloric intake in obese subjects; however, no changes were found in fasting glucose, insulin, and ghrelin levels. A significant rise has also been observed in abundance of *Bifidobacterium* spp. in mice with diet-induced (Dewulf et al. 2011) or genetically determined obese (*ob/ob*) model (Cani et al. 2009b), which was fortified with insulin-type fructans. In addition, the latter group also reported improved gut barrier and tight junction integrity and mucosal health. In a recent study, Angelino et al. (2019) reported synbiotic mixture in pasta (prebiotic barley β-glucans and probiotic *Bacillus coagulans*) improved plasma hs-CRP, plasma LDL/HDL cholesterol ratio, and sedentary overweight and obese adults and also improved microbiome profile.

Thus, the use of probiotics alone or in combination with prebiotics (i.e., synbiotics) to manage the obesity restoring "healthy signature gut microbiome" could be one effective approach alongside other management routes. Nevertheless, more mechanistic studies are required to explore the definitive pathways of probiotic action. If we understand the mechanisms by which probiotics affect adipose tissue, serum lipids, satiety center, and other neuroendocrine factors associated with general metabolism, it will lead to identifying new and effective probiotic, prebiotic, or synbiotic management strategies.

9.6.4 Role of Probiotics in Gastrointestinal (GI) Diseases

Though many of the disorders of the GI tract occur due to infections, some metabolic disorders have also certain connections with this organ. Some of them are also chronic and difficult to diagnose which include irritable bowel syndrome, lactose intolerance, GERD, celiac disease, and peptic ulcer. Moreover, the GI tract is the site of origin and the hotspot for many metabolic disorders. To maintain a healthy intestinal tract, it needs a vast microbial colony. Still, the GI tract suffers from different inflammatory diseases due to the modification of the microbial population (Sartor 2008). After the ingestion of oral probiotics, the mucosal and systemic immunity of the GI tract becomes stronger, and also the nutritional and microbial balance of the intestine gets improved (Fuller 1989). Epithelial cells along with gut microflora form an effective barrier to the harmful pathogens. Still, diseases occur on the disturbance in this barrier due to stress, illness, antibiotic treatment, or change in diet (Schmidt and Cohn 2001). Some studies have suggested that probiotics are very useful for some GI diseases like ulcerative colitis and pouchitis (Venturi et al. 1999). It was found that the fecal concentration of *Lactobacillus*, *Bifidobacterium*, and *S. thermophilus* was markedly increased, and after stopping probiotic consumption, microbial concentration was back to their baseline. No toxic reaction was found during the treatment (Friedman and George 2000).

Lactose intolerance is mainly caused by lactase deficiency. It was reported that lactose is hydrolyzed into glucose and galactose from yogurt than from the milk in lactose-deficient individuals (Marteau et al. 1997).

The mechanism of how probiotics are effective for GI diseases still remains controversial. One of the main reasons is the competition for the available receptor in between pathogen and probiotics and the secretion of some antimicrobial peptide by them (Ahn and Stiles 1990). Probiotics help in the secretion of mucin which in turn prevents the harmful pathogen to bind with the epithelial receptors. Oral administration of *Lactobacillus GG* induces an anti-inflammatory, Th-2 mediated systemic immune response. It also enhances the immunological functions in the GI tract (Malin et al. 1996). However, the above-said mechanism needed many more clinical trials to be proven.

9.7 Conclusion

In recent years, ample shreds of evidence have been accumulated that unequivocally reflect that some specialized bacteria can regulate and maintain the parameters related to various metabolic disorders. In human, *Lactobacillus* mainly target the small intestine while *Bifidobacterium* the colon for probiotic action. Both the intestinal sites respond differently to the type of bacteria and specific metabolites produced, though their mode of actions is different. Gut microbiota can alter metabolic, neurohormonal, immune pathways associated with metabolic disorders. Consequently, some carefully selected dietary strategies are emerging, to target the gut ecosystem, as an important tool to control metabolic diseases.

Probiotics are a type of functional food that can stabilize the intestinal microbial environment and the mucosal barrier to maintain immune responses to compete with pathologic bacteria and also for downregulating adverse immune reactions. It generally shares some beneficial properties like improved gut barrier function, reduced hepatic inflammation, etc. The effect of probiotics provides good management of such metabolic diseases, primarily T2DM, CVD, and obesity. Moreover, probiotics can also serve as a growth stimulus for natural probiotic strain, improve the survivability of probiotic bacteria, and provide beneficial effects to the host.

Metabolic disorders, particularly those originating from altered lifestyles, have been correlated with an increased risk of morbidity and mortality. Growing knowledge from the arenas, like gut microbiology, microbiomics, and metabolomics, suggests that gut microbiota plays a significant role in the initiation and progression of metabolic disorders. Several lines of evidence revealed that the gut microbiota could modulate plasma glucose, appetite, serum lipids, and inflammatory reactions. Among scientific communities, there is enough consensus in this context and accepts that the gut microbiota composition and function can be modulated by probiotics and prebiotics. In the past two decades, numerous studies have also unwrapped that probiotics and prebiotics have positive effects on T2DM and CVDs by re-patterning gut microbiota, regulating insulin signaling, lowering serum cholesterol level, and lowering the grade of intestinal inflammation. However, elucidating the regulatory points of host metabolic pathways that the microbes tweak and the interactions between intestinal microbiota and ingested probiotics continues to present a challenge for the forthcoming years. In addition, more specific and thoroughly designed (in vivo*)* clinical trials addressing the efficacy and efficiency of current or potential treatments on therapeutic applications in metabolic disorders are also needed to eliminate uncertainties, if any.

9.8 Future Directions

As discussed earlier, ethnic and traditional uses of probiotics and prebiotics are virtually millennia-old practice. However, the same threads of benefits are now being weaved by vigorous, reproducible scientific researches and testimonials. So this can be stated as there is a paradigm shift from generic uses of these probiotics and prebiotics to target-oriented functional foods for prevention or treatment of definite anomaly, cutting down potential adverse reactions. Nowadays, we use some probiotics (*Lactobacillus*, *Bifidobacterium*, *Lactococcus*, etc.) and prebiotics (fructans, inulin, lactosucrose, oligofructose, etc.) in our daily food habit (Yoo and Kim 2016; Markowiak and Ślizewska 2017). Probiotics and prebiotics are highly acceptable due to their low harmful effects, in general, although there are no standard guidelines to consume probiotics and prebiotics in human cases (Yoo and Kim 2016). Besides the most highlighted microbiota that has the probiotic effect on host metabolism, recently several other taxa, such as *Saccharomyces cerevisiae*, *Enterococcus faecium*, *Enterobacter halii*, and *Akkermansia muciniphila*, have been identified and shown the novel mechanism of action for obesity (Cani and Van Hul 2015). Nowadays, research on next-generation probiotics is going on to control obesity and related metabolic disorders. In the last few years, some notable advancements are made rapidly in genomics, proteomics, metabolomics, nutrigenomics, and the progression of ongoing human microbiome projects. Whole-genome sequences of several proven commercial probiotic strains are available to generate information that has widen the scopes and prospects of probiotics as natural food supplements for the management of different metabolic disorders. These will be very useful in search of next-generation probiotics that target metabolic syndrome and related disorders. Though many of these probiotics, prebiotics, or synbiotics are in clinical trials with some promising results, still, more broad-scale clinical studies with subjects from different age group, sex, and geographical regions are wanting. Alongside, standard safety guidelines regarding the specific administration of probiotics and prebiotics in human cases are also needed.

References

Abbasi B, Ghiasvand R, Mirlohi M (2017) Kidney function improvement by soy milk containing *Lactobacillus plantarum* A7 in type 2 diabetic patients with nephropathy: a double-blinded randomized controlled trial. Iran J Kidney Dis 11:36

Abbasi B, Mirlohi M, Daniali M, Ghiasvand R (2018) Effects of probiotic soy milk on lipid panel in type 2 diabetic patients with nephropathy: a double-blind randomized clinical trial. Prog Nutr. https://doi.org/10.23751/pn.v20i2-S.5342

Abd El-Gawad IA, El-Sayed EM, Hafez SA et al (2005) The hypocholesterolaemic effect of milk yoghurt and soy-yoghurt containing Bifidobacteria in rats fed on a cholesterol-enriched diet. Int Dairy J 15:37. https://doi.org/10.1016/j.idairyj.2004.06.001

Ahn C, Stiles ME (1990) Antibacterial activity of lactic acid bacteria isolated from vacuum-packaged meats. J Appl Bacteriol 69:302. https://doi.org/10.1111/j.1365-2672.1990.tb01520.x

Alissa EM, Ferns GA (2012) Functional foods and nutraceuticals in the primary prevention of cardiovascular diseases. J Nutr Metab 2012:569486

Alkhatib A, Tsang C, Tiss A et al (2017) Functional foods and lifestyle approaches for diabetes prevention and management. Nutrients 9:1310

Al-Lahham SH, Peppelenbosch MP, Roelofsen H et al (2010) Biological effects of propionic acid in humans; metabolism, potential applications and underlying mechanisms. Biochim Biophys Acta 1801:1175

Allison DB, Kaprio J, Korkeila M et al (1996) The heritability of body mass index among an international sample of monozygotic twins reared apart. Int J Obes Relat Metab Disord 20:501

Angelino D, Martina A, Rosi A et al (2019) Glucose- and lipid-related biomarkers are affected in healthy obese or hyperglycemic adults consuming a whole-grain pasta enriched in prebiotics and probiotics: a 12-week randomized controlled trial. J Nutr 149:1714. https://doi.org/10.1093/jn/nxz071

Annuk H, Shchepetova J, Kullisaar T et al (2003) Characterization of intestinal lactobacilli as putative probiotic candidates. J Appl Microbiol 94:403. https://doi.org/10.1046/j.1365-2672.2003.01847.x

Arneth BM (2018) Gut-brain axis biochemical signalling from the gastrointestinal tract to the central nervous system: gut dysbiosis and altered brain function. Postgrad Med J 94:446. https://doi.org/10.1136/postgradmedj-2017-135424

Balmer SE, Wharton BA (1989) Diet and feacal flora in the newborn: breast milk and infant formula. Arch Dis Child 64:1672. https://doi.org/10.1136/adc.64.12.1672

Behera SS, Ray RC, Zdolec N (2018) Lactobacillus plantarum with functional properties: an approach to increase safety and shelf-life of fermented foods. Biomed Res Int 2018. https://doi.org/10.1155/2018/9361614

Benn AML, Heng NCK, Broadbent JM, Thomson WM (2018) Studying the human oral microbiome: challenges and the evolution of solutions. Aust Dent J 63:14

Bennett BJ, Vallim TQDA, Wang Z et al (2013) Trimethylamine-N-Oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. Cell Metab 17:49. https://doi.org/10.1016/j.cmet.2012.12.011

Bindels LB, Dewulf EM, Delzenne NM (2013) GPR43/FFA2: physiopathological relevance and therapeutic prospects. Trends Pharmacol Sci 34:226

Bindels LB, Delzenne NM, Cani PD, Walter J (2015) Opinion: towards a more comprehensive concept for prebiotics. Nat Rev Gastroenterol Hepatol 12:303

Bordenstein SR, Theis KR (2015) Host biology in light of the microbiome: ten principles of holobionts and hologenomes. PLoS Biol 18:e1002226. https://doi.org/10.1371/journal.pbio.1002226

Boulangé CL, Neves AL, Chilloux J et al (2016) Impact of the gut microbiota on inflammation, obesity, and metabolic disease. Genome Med 8:42

Brashears MM, Gilliland SE, Buck LM (1998) Bile salt deconjugation and cholesterol removal from media by *Lactobacillus casei*. J Dairy Sci 81:2103. https://doi.org/10.3168/jds.S0022-0302(98)75785-6

Brunkwall L, Orho-Melander M (2017) The gut microbiome as a target for prevention and treatment of hyperglycaemia in type 2 diabetes: from current human evidence to future possibilities. Diabetologia 60:943

Burleigh MC, Liddle L, Monaghan C et al (2018) Salivary nitrite production is elevated in individuals with a higher abundance of oral nitrate-reducing bacteria. Free Radic Biol Med 120:80. https://doi.org/10.1016/j.freeradbiomed.2018.03.023

Buse JB, DeFronzo RA, Rosenstock J et al (2016) The primary glucose-lowering effect of metformin resides in the gut, not the circulation: results from short-term pharmacokinetic and 12-week dose-ranging studies. Diabetes Care 39:198. https://doi.org/10.2337/dc15-0488

Canfora EE, Jocken JW, Blaak EE (2015) Short-chain fatty acids in control of body weight and insulin sensitivity. Nat Rev Endocrinol 11:577

Cani PD (2014) Metabolism in 2013: the gut microbiota manages host metabolism. Nat Rev Endocrinol 10:74

Cani PD, Van Hul M (2015) Novel opportunities for next-generation probiotics targeting metabolic syndrome. Curr Opin Biotechnol 32:21

Cani PD, Amar J, Iglesias MA et al (2007) Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 56:1761. https://doi.org/10.2337/db06-1491

Cani PD, Bibiloni R, Knauf C et al (2008) Changes in gut microbiota control metabolic diet–induced obesity and diabetes in mice. Diabetes 57:1470. https://doi.org/10.2337/db07-1403. Additional

Cani PD, Lecourt E, Dewulf EM et al (2009a) Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. Am J Clin Nutr 90:1236. https://doi.org/10.3945/ajcn.2009.28095

Cani PD, Possemiers S, Van De Wiele T et al (2009b) Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 58:1091. https://doi.org/10.1136/gut.2008.165886

Cani PD, Osto M, Geurts L, Everard A (2012) Involvement of gut microbiota in the development of low-grade inflammation and type 2 diabetes associated with obesity. Gut Microbes 3:279

Cencic A, Chingwaru W (2010) The role of functional foods, nutraceuticals, and food supplements in intestinal health. Nutrients 2:611

Cerdó T, García-Santos JA, Bermúdez MG, Campoy C (2019) The role of probiotics and prebiotics in the prevention and treatment of obesity. Nutrients 11:635

Chen CC, Walker WA (2005) Probiotics and prebiotics: role in clinical disease states. Adv Pediatr 52:77. https://doi.org/10.1016/j.yapd.2005.03.001

Cífková R, Krajčoviechová A (2015) Dyslipidemia and cardiovascular disease in women. Curr Cardiol Rep 17:609

Collins S, Reid G (2016) Distant site effects of ingested prebiotics. Nutrients 8:523

Corthésy B, Gaskins HR, Mercenier A (2007) Cross-talk between probiotic bacteria and the host immune system. J Nutr 137:781S

Cotillard A, Kennedy SP, Kong LC et al (2013) Dietary intervention impact on gut microbial gene richness. Nature 500:585. https://doi.org/10.1038/nature12480

Cruchet S, Furnes R, Maruy A et al (2015) The use of probiotics in pediatric gastroenterology: a review of the literature and recommendations by Latin-American experts. Pediatr Drugs 17:199

Cryan JF, O'riordan KJ, Cowan CSM et al (2019) The microbiota-gut-brain axis. Physiol Rev 99: 1877. https://doi.org/10.1152/physrev.00018.2018

Czerucka D, Piche T, Rampal P (2007) Review article: yeast as probiotics - Saccharomyces boulardii. Aliment Pharmacol Ther 26:767

Davani-Davari D, Negahdaripour M, Karimzadeh I et al (2019) Prebiotics: definition, types, sources, mechanisms, and clinical applications. Foods 8:92

DeBoer MD (2013) Obesity, systemic inflammation, and increased risk for cardiovascular disease and diabetes among adolescents: a need for screening tools to target interventions. Nutrition 29: 379

Delzenne NM, Cani PD, Neyrinck AM (2007) Modulation of glucagon-like peptide 1 and energy metabolism by inulin and oligofructose: experimental data. J Nutr 137:2547S

Delzenne NM, Neyrinck AM, Bäckhed F, Cani PD (2011) Targeting gut microbiota in obesity: effects of prebiotics and probiotics. Nat Rev Endocrinol 7:639

Dewhirst FE, Chen T, Izard J et al (2010) The human oral microbiome. J Bacteriol 192:5002. https://doi.org/10.1128/JB.00542-10

Dewulf EM, Cani PD, Neyrinck AM et al (2011) Inulin-type fructans with prebiotic properties counteract GPR43 overexpression and PPARγ-related adipogenesis in the white adipose tissue of high-fat diet-fed mice. J Nutr Biochem 22:712. https://doi.org/10.1016/j.jnutbio.2010.05.009

DiBaise JK, Zhang H, Crowell MD et al (2008) Gut microbiota and its possible relationship with obesity. Mayo Clin Proc 83:460. https://doi.org/10.4065/83.4.460

Doel JJ, Benjamin N, Hector MP et al (2005) Evaluation of bacterial nitrate reduction in the human oral cavity. Eur J Oral Sci 113:14. https://doi.org/10.1111/j.1600-0722.2004.00184.x

Donohoe DR, Garge N, Zhang X et al (2011) The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. Cell Metab 13:517. https://doi.org/10.1016/j.cmet.2011.02.018

Egan BM, Li J, Qanungo S, Wolfman TE (2013) Blood pressure and cholesterol control in hypertensive hypercholesterolemic patients: national health and nutrition examination surveys 1988-2010. Circulation 28:29. https://doi.org/10.1161/CIRCULATIONAHA.112.000500

Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A et al (2011) Effect of probiotic yogurt containing *Lactobacillus acidophilus* and *Bifidobacterium lactis* on lipid profile in individuals with type 2 diabetes mellitus. J Dairy Sci 94:3288. https://doi.org/10.3168/jds.2010-4128

Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A et al (2012) Probiotic yogurt improves antioxidant status in type 2 diabetic patients. Nutrition 28:539. https://doi.org/10.1016/j.nut.2011.08.013

Ettinger G, MacDonald K, Reid G, Burton JP (2014) The influence of the human microbiome and probiotics on cardiovascular health. Gut Microbes 5:719

Everard A, Lazarevic V, Derrien 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: 2775–2786

FAO/WHO (2002) Guidelines for the evaluation of probiotics in food (Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food). Food and Agricultural Organization of the United Nations. World Health Organization. https://doi.org/10.1111/j.1469-0691.2012.03873

Feinberg AP, Irizarry RA, Fradin D et al (2011) Personalized epigenomic signatures that are stable over time and covary with Body Mass Index. Sci Transl Med 3:65er1

Forslund K, Hildebrand F, Nielsen T et al (2015) Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. Nature 528:262. https://doi.org/10.1038/nature15766

Frank DN, Robertson CE, Hamm CM et al (2011) Disease phenotype and genotype are associated with shifts in intestinal-associated microbiota in inflammatory bowel diseases. Inflamm Bowel Dis 17:179. https://doi.org/10.1002/ibd.21339

Friedman G, George J (2000) Treatment of refractory "Pouchitis" with prebiotic and probiotic therapy. Gastroenterology. https://doi.org/10.1016/s0016-5085(00)85255-5

Frydrych LM, Bian G, O'Lone DE et al (2018) Obesity and type 2 diabetes mellitus drive immune dysfunction, infection development, and sepsis mortality. J Leukoc Biol 104:525

Fukushima M, Yamada A, Endo T, Nakano M (1999) Effects of a mixture of organisms, *Lactobacillus acidophilus* or *Streptococcus faecalis* on δ6-desaturase activity in the livers of rats fed a fat- and cholesterol-enriched diet. Nutrition 15:373. https://doi.org/10.1016/S0899-9007(99)00030-1

Fuller R (1989) Probiotics in man and animals: a review. J Appl Bacteriol 66:365

Gall WE, Beebe K, Lawton KA et al (2010) α-hydroxybutyrate is an early biomarker of insulin resistance and glucose intolerance in a nondiabetic population. PLoS One 5:e10883. https://doi.org/10.1371/journal.pone.0010883

Gao Z, Yin J, Zhang J et al (2009) Butyrate improves insulin sensitivity and increases energy expenditure in mice. Diabetes 58:1509. https://doi.org/10.2337/db08-1637

Gargano LM, Hughes JM (2014) Microbial origins of chronic diseases. Annu Rev Public Health 35:65

Gargari BP, Dehghan P, Aliasgharzadeh A, Jafar-Abadi MA (2013) Effects of high performance inulin supplementation on glycemic control and antioxidant status in women with type 2 diabetes. Diabetes Metab J 37:140. https://doi.org/10.4093/dmj.2013.37.2.140

Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125:1401

Gibson GR, Probert HM, Van Loo J et al (2004) Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr Res Rev 17:259. https://doi.org/10.1079/nrr200479

Gill SR, Pop M, DeBoy RT et al (2006) Metagenomic analysis of the human distal gut microbiome. Science 312:1355. https://doi.org/10.1126/science.1124234

Gill PA, van Zelm MC, Muir JG, Gibson PR (2018) Review article: short chain fatty acids as potential therapeutic agents in human gastrointestinal and inflammatory disorders. Aliment Pharmacol Ther 48:15

Go A, Mozaffarian D et al (2013) Statistical fact sheet 2013 update nutrition & cardiovascular diseases nutrition & CVD - 2013 statistical fact sheet. Circulation 127:e62

Granato D, Branco GF, Cruz AG et al (2010) Probiotic dairy products as functional foods. Compr Rev Food Sci Food Saf 9:455. https://doi.org/10.1111/j.1541-4337.2010.00120.x

Grover S, Mallappa R, Rokana N et al (2012) Management of metabolic syndrome through probiotic and prebiotic interventions. Indian J Endocrinol Metab 16:20. https://doi.org/10.4103/2230-8210.91178

Grundy SM (2008) Promise of low-density lipoprotein–lowering therapy for primary and secondary prevention. Circulation 117:569. https://doi.org/10.1161/circulationaha.107.720300

Guardamagna O, Amaretti A, Puddu PE et al (2014) Bifidobacteria supplementation: effects on plasma lipid profiles in dyslipidemic children. Nutrition 30:831. https://doi.org/10.1016/j.nut.2014.01.014

Gul K, Singh AK, Jabeen R (2016) Nutraceuticals and functional foods: the foods for the future world. Crit Rev Food Sci Nutr 56:2617

Guo Z, Liu XM, Zhang QX et al (2011) Influence of consumption of probiotics on the plasma lipid profile: a meta-analysis of randomised controlled trials. Nutr Metab Cardiovasc Dis 21:844

Gur J, Mawuntu M, Martirosyan D (2018) FFC's advancement of functional food definition. Funct Foods Heal Dis. https://doi.org/10.31989/ffhd.v8i7.531

Guyton JR (2006) Benefit versus risk in statin treatment. Am J Cardiol 97:95C. https://doi.org/10.1016/j.amjcard.2005.12.016

Hardy G (2000) Nutraceuticals and functional foods: introduction and meaning. Nutrition 16:688

He M, Shi B (2017) Gut microbiota as a potential target of metabolic syndrome: the role of probiotics and prebiotics. Cell Biosci 7:54

Heidemann C, Schulze MB, Franco OH et al (2008) Dietary patterns and risk of mortality from cardiovascular disease, cancer, and all causes in a prospective cohort of women. Circulation 118:230. https://doi.org/10.1161/CIRCULATIONAHA.108.771881

Hezel MP, Weitzberg E (2015) The oral microbiome and nitric oxide homoeostasis. Oral Dis 21:7. https://doi.org/10.1111/odi.12157

Honey K (2008) Good bugs, bad bugs: learning what we can from the microorganisms that colonize our bodies. J Clin Invest 118:3817

Howell TH (1988) Metchnikoff and prolongation of life. Age Ageing 17:420. https://doi.org/10.1093/ageing/17.6.420

Hur KY, Lee MS (2015) Gut microbiota and metabolic disorders. Diabetes Metab J 39:198. https://doi.org/10.4093/dmj.2015.39.3.198

Huttenhower C, Gevers D, Knight R et al (2012) Structure, function and diversity of the healthy human microbiome. Nature 486:207. https://doi.org/10.1038/nature11234

Hyde ER, Andrade F, Vaksman Z et al (2014) Metagenomic analysis of nitrate-reducing bacteria in the oral cavity: implications for nitric oxide homeostasis. PLoS One 9:e88645. https://doi.org/10.1371/journal.pone.0088645

Institute of Health Metrics and Evaluation (2019) GBD results tool. Institue Heal, Metrics Eval

Ipar N, Aydogdu SD, Yildirim GK et al (2015) Effects of synbiotic on anthropometry, lipid profile and oxidative stress in obese children. Benef Microbes 6:775. https://doi.org/10.3920/BM2015.0011

James WPT (2008) The epidemiology of obesity: the size of the problem. J Intern Med 263:336

Jia Q, Li H, Zhou H et al (2019) Role and effective therapeutic target of gut microbiota in heart failure. Cardiovasc Ther 2019:5164298. https://doi.org/10.1155/2019/5164298

Jones ML, Martoni CJ, Prakash S (2012) Cholesterol lowering and inhibition of sterol absorption by Lactobacillus reuteri NCIMB 30242: a randomized controlled trial. Eur J Clin Nutr 66:1234. https://doi.org/10.1038/ejcn.2012.126

Kadooka Y, Sato M, Imaizumi K et al (2010) Regulation of abdominal adiposity by probiotics (Lactobacillus gasseri SBT2055) in adults with obese tendencies in a randomized controlled trial. Eur J Clin Nutr 64:636. https://doi.org/10.1038/ejcn.2010.19

Kalliomäki M, Collado MC, Salminen S, Isolauri E (2008) Early differences in faecal microbiota composition in children may predict later weight gain. Am J Clin Nutr 87:534

Kapil V, Haydar SMA, Pearl V et al (2013) Physiological role for nitrate-reducing oral bacteria in blood pressure control. Free Radic Biol Med 55:93. https://doi.org/10.1016/j.freeradbiomed.2012.11.013

Kasubuchi M, Hasegawa S, Hiramatsu T et al (2015) Dietary gut microbial metabolites, short-chain fatty acids, and host metabolic regulation. Nutrients 7:2839

Kawamoto S, Tran TH, Maruya M et al (2012) The inhibitory receptor PD-1 regulates IgA selection and bacterial composition in the gut. Science 336:485. https://doi.org/10.1126/science.1217718

Key T, Davey G (1996) Prevalence of obesity is low in people who do not eat meat. Br Med J 313:816

Kilian M, Chapple ILC, Hannig M et al (2016) The oral microbiome - an update for oral healthcare professionals. Br Dent J 221:657. https://doi.org/10.1038/sj.bdj.2016.865

Kim M, Shin HK (1998) The water-soluble extract of chicory influences serum and liver lipid concentrations, cecal short-chain fatty acid concentrations and fecal lipid excretion in rats. J Nutr 128:1731. https://doi.org/10.1093/jn/128.10.1731

Kim SJ, Park SH, Sin HS et al (2017) Hypocholesterolemic effects of probiotic mixture on diet-induced hypercholesterolemic rats. Nutrients 9:293. https://doi.org/10.3390/nu9030293

Kimoto H, Ohmomo S, Okamoto T (2002) Cholesterol removal from media by lactococci. J Dairy Sci 85:3182. https://doi.org/10.3168/jds.S0022-0302(02)74406-8

Kitai T, Tang WHW (2018) Gut microbiota in cardiovascular disease and heart failure. Clin Sci 132:85

Koeth RA, Wang Z, Levison BS et al (2013) Intestinal microbiota metabolism of l-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat Med 19:576. https://doi.org/10.1038/nm.3145

Koropatkin NM, Cameron EA, Martens EC (2012) How glycan metabolism shapes the human gut microbiota. Nat Rev Microbiol 10:323

Kullisaar T, Zilmer M, Mikelsaar M et al (2002) Two antioxidative lactobacilli strains as promising probiotics. Int J Food Microbiol 72:215. https://doi.org/10.1016/S0168-1605(01)00674-2

Kumar M, Nagpal R, Kumar R et al (2012) Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases. Exp Diabetes Res 2012:902917

Kyriachenko Y, Falalyeyeva T, Korotkyi O et al (2019) Crosstalk between gut microbiota and antidiabetic drug action. World J Diabetes 10:154. https://doi.org/10.4239/wjd.v10.i3.154

Lam V, Su J, Koprowski S et al (2012) Intestinal microbiota determine severity of myocardial infarction in rats. FASEB J 26:1727. https://doi.org/10.1096/fj.11-197921

Larsen N, Vogensen FK, Van Den Berg FWJ et al (2010) Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. PLoS One 5:e9085. https://doi.org/10.1371/journal.pone.0009085

Le Barz M, Daniel N, Varin TV 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. FASEB J 33:4921. https://doi.org/10.1096/fj.201801672R

Lebeer S, Vanderleyden J, De Keersmaecker SCJ (2010) Host interactions of probiotic bacterial surface molecules: comparison with commensals and pathogens. Nat Rev Microbiol 8:171

Lee SJ, Bose S, Seo JG et al (2014) The effects of co-administration of probiotics with herbal medicine on obesity, metabolic endotoxemia and dysbiosis: a randomized double-blind controlled clinical trial. Clin Nutr 33:973. https://doi.org/10.1016/j.clnu.2013.12.006

Lewis SJ, Burmeister S (2005) A double-blind placebo-controlled study of the effects of *Lactobacillus acidophilus* on plasma lipids. Eur J Clin Nutr 59:776. https://doi.org/10.1038/sj.ejcn.1602139

Ley RE, Turnbaugh PJ, Klein S, Gordon JI (2006) Microbial ecology: human gut microbes associated with obesity. Nature 444:1022. https://doi.org/10.1038/4441022a

Liang X, Zhang Z, Lv Y et al (2020) Reduction of intestinal trimethylamine by probiotics ameliorated lipid metabolic disorders associated with atherosclerosis. Nutrition 79–80: 110941. https://doi.org/10.1016/j.nut.2020.110941

Ling C, Rönn T (2019) Epigenetics in human obesity and type 2 diabetes. Cell Metab 29:1028

Lloyd-Price J, Abu-Ali G, Huttenhower C (2016) The healthy human microbiome. Genome Med 8: 51

Lye HS, Rahmat-Ali GR, Liong MT (2010a) Mechanisms of cholesterol removal by lactobacilli under conditions that mimic the human gastrointestinal tract. Int Dairy J 20:169. https://doi.org/10.1016/j.idairyj.2009.10.003

Lye HS, Rusul G, Liong MT (2010b) Removal of cholesterol by lactobacilli via incorporation and conversion to coprostanol. J Dairy Sci 93:1383. https://doi.org/10.3168/jds.2009-2574

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. Ann Nutr Metab 40:137. https://doi.org/10.1159/000177907

Marchesi JR, Adams DH, Fava F et al (2016) The gut microbiota and host health: a new clinical frontier. Gut 65:330. https://doi.org/10.1136/gutjnl-2015-309990

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

Marteau P, Vesa T, Rambaud JC (1997) Lactose maldigestion. In: Probiotics 2. Springer, Dordrecht, pp 65–88

Mårtensson O, Biörklund M, Lambo AM et al (2005) Fermented, ropy, oat-based products reduce cholesterol levels and stimulate the bifidobacteria flora in humans. Nutr Res. https://doi.org/10.1016/j.nutres.2005.03.004

Martínez JA, Milagro FI, Claycombe KJ, Schalinske KL (2014) Epigenetics in adipose tissue, obesity, weight loss, and diabetes. Adv Nutr 5:71. https://doi.org/10.3945/an.113.004705

Martirosyan DM, Singh J (2015) A new definition of functional food by FFC: what makes a new definition unique? Funct Foods Heal Dis 5:209. https://doi.org/10.31989/ffhd.v5i6.183

Marzoa-Rivas R, Crespo-Leiro MG, Paniagua-Marin MJ et al (2005) Safety of statins when response is carefully monitored: a study of 336 heart recipients. Transplant Proc 37:4071

Mattila-Sandholm T, Myllärinen P, Crittenden R et al (2002) Technological challenges for future probiotic foods. Int Dairy J:173–182
Mayer EA, Tillisch K, Gupta A (2015) Gut/brain axis and the microbiota. J Clin Invest 125:926
McAllister EJ, Dhurandhar NV, Keith SW et al (2009) Ten putative contributors to the obesity epidemic. Crit Rev Food Sci Nutr 49:868. https://doi.org/10.1080/10408390903372599
McClements DJ (2019) Future foods: how modern science is transforming the way we eat. Springer
McCracken K, Phillips DR (2017) Global health: an introduction to current and future trends, 2nd edn, pp 1–437
McEvoy CT, Temple N, Woodside JV (2012) Vegetarian diets, low-meat diets and health: a review. Public Health Nutr 15:2287
McNabney SM, Henagan TM (2017) Short chain fatty acids in the colon and peripheral tissues: a focus on butyrate, colon cancer, obesity and insulin resistance. Nutrients 9:1348
Mengual L, Roura P, Serra M et al (2010) Multifactorial control and treatment intensity of type-2 diabetes in primary care settings in Catalonia. Cardiovasc Diabetol 9:14. https://doi.org/10.1186/1475-2840-9-14
Meydani SN, Ha WK (2000) Immunologic effects of yogurt. Am J Clin Nutr 71:861
Micha R, Wallace SK, Mozaffarian D (2010) Red and processed meat consumption and risk of incident coronary heart disease, stroke, and diabetes mellitus: a systematic review and meta-analysis. Circulation 121:2271. https://doi.org/10.1161/CIRCULATIONAHA.109.924977
Mirmiran P (2014) Functional foods-based diet as a novel dietary approach for management of type 2 diabetes and its complications: a review. World J Diabetes 5:267. https://doi.org/10.4239/wjd.v5.i3.267
Mitsuoka T (2014) Development of functional foods. Biosci Microbiota Food Health 33:117
Mohamadshahi M, Veissi M, Haidari F et al (2014) Effects of probiotic yogurt consumption on lipid profile in type 2 diabetic patients: a randomized controlled clinical trial. J Res Med Sci 19:531
Musso G, Gambino R, Cassader M (2010) Obesity, diabetes, and gut microbiota: the hygiene hypothesis expanded? Diabetes Care 33:2277
Nadal I, Santacruz A, Marcos A et al (2009) Shifts in clostridia, bacteroides and immunoglobulin-coating fecal bacteria associated with weight loss in obese adolescents. Int J Obes 33:758. https://doi.org/10.1038/ijo.2008.260
Nagatomo Y, Tang WHW (2015) Intersections between microbiome and heart failure: revisiting the gut hypothesis. J Card Fail 21:973
Nagpal R, Kumar A, Kumar M et al (2012) Probiotics, their health benefits and applications for developing healthier foods: a review. FEMS Microbiol Lett 334:1
Nguyen TDT, Kang JH, Lee MS (2007) Characterization of Lactobacillus plantarum PH04, a potential probiotic bacterium with cholesterol-lowering effects. Int J Food Microbiol 113:358. https://doi.org/10.1016/j.ijfoodmicro.2006.08.015
Ooi LG, Liong MT (2010) Cholesterol-lowering effects of probiotics and prebiotics: a review of in vivo and in vitro findings. Int J Mol Sci 11:2499
Panagiotakos D, Bountziouka V, Zeimbekis A et al (2007) Food pattern analysis and prevalence of cardiovascular disease risk factors among elderly people from Mediterranean islands. J Med Food 10:615. https://doi.org/10.1089/jmf.2007.414
Panwar H, Rashmi HM, Batish VK, Grover S (2013) Probiotics as potential biotherapeutics in the management of type 2 diabetes - prospects and perspectives. Diabetes Metab Res Rev 29:103
Parnell JA, Reimer RA (2009) Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. Am J Clin Nutr 89:1751. https://doi.org/10.3945/ajcn.2009.27465
Pei R, Martin DA, DiMarco DM, Bolling BW (2017) Evidence for the effects of yogurt on gut health and obesity. Crit Rev Food Sci Nutr 57:1569. https://doi.org/10.1080/10408398.2014.883356
Pérez-Díaz IM (2019) Fermented vegetables as vectors for relocation of microbial diversity from the environment tothe human gut. In: How fermented foods feed a healthy gut microbiota: a nutrition continuum, pp 91–123

Peterson J, Garges S, Giovanni M et al (2009) The NIH human microbiome project. Genome Res. https://doi.org/10.1101/gr.096651.109
Phieler J, Garcia-Martin R, Lambris JD, Chavakis T (2013) The role of the complement system in metabolic organs and metabolic diseases. Semin Immunol 25:47
Pintado MM, Gomes AM, Freitas AC (2014) Probiotics and their therapeutic role. Probiotic Bact Fundam Ther Technol Asp 47–93. https://doi.org/10.4032/9789814411639
Plana N, Nicolle C, Ferre R et al (2008) Plant sterol-enriched fermented milk enhances the attainment of LDL-cholesterol goal in hypercholesterolemic subjects. Eur J Nutr 47:32. https://doi.org/10.1007/s00394-007-0693-4
Poirier P, Giles TD, Bray GA et al (2006) Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss. Arterioscler Thromb Vasc Biol 26:968
Purdham DM, Zou MX, Rajapurohitam V, Karmazyn M (2004) Rat heart is a site of leptin production and action. Am J Physiol Heart Circ Physiol 287:H2877. https://doi.org/10.1152/ajpheart.00499.2004
Qin J, Li R, Raes J et al (2010) A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464:59. https://doi.org/10.1038/nature08821
Raman R, Thomas RG, Weiner MW et al (2010) Diversity of the human intestinal microbial flora. Science 308:1635. https://doi.org/10.1097/WAD.0b013e3181aba588.MRI
Rawls JF, Mahowald MA, Ley RE, Gordon JI (2006) Reciprocal gut microbiota transplants from zebrafish and mice to germ-free recipients reveal host habitat selection. Cell 127:423. https://doi.org/10.1016/j.cell.2006.08.043
Razmpoosh E, Javadi A, Ejtahed HS et al (2019) The effect of probiotic supplementation on glycemic control and lipid profile in patients with type 2 diabetes: a randomized placebo controlled trial. Diabetes Metab Syndr Clin Res Rev 13:175. https://doi.org/10.1016/j.dsx.2018.08.008
Re RN (2009) Obesity-related hypertension. Ochsner J 9:133
Reid G, Younes JA, Van Der Mei HC et al (2011) Microbiota restoration: natural and supplemented recovery of human microbial communities. Nat Rev Microbiol 9:27
Reimer RA, Russell JC (2008) Glucose tolerance, lipids, and GLP-1 secretion in JCR:LA-cp rats fed a high protein fiber diet. Obesity 16:40. https://doi.org/10.1038/oby.2007.16
Riccardi G, Capaldo B, Vaccaro O (2005) Functional foods in the management of obesity and type 2 diabetes. Curr Opin Clin Nutr Metab Care 8:630
Riveros-McKay F, Mistry V, Bounds R et al (2019) Genetic architecture of human thinness compared to severe obesity. PLoS Genet 15:e1007603. https://doi.org/10.1371/journal.pgen.1007603
Roberfroid MB (2000) Prebiotics and probiotics: are they functional foods? Am J Clin Nutr 71: 1682S
Roberfroid M (2007) Prebiotics: the concept revisited. J Nutr 137:830S
Rogers GB, Keating DJ, Young RL et al (2016) From gut dysbiosis to altered brain function and mental illness: mechanisms and pathways. Mol Psychiatry 21:738
Rokhsefat S, Lin A, Comelli EM (2016) Mucin–microbiota interaction during postnatal maturation of the intestinal ecosystem: clinical implications. Dig Dis Sci 61:1473
da Rosa EM, Scatola RP, Possa R (2008) Cardiovascular risk in vegetarians and omnivores: a comparative study. Arq Bras Cardiol 91:263
Rosenberg E, Zilber-Rosenberg I (2013) The hologenome concept: human, animal and plant microbiota, pp 1–178
Ruscica M, Pavanello C, Gandini S et al (2018) Effect of soy on metabolic syndrome and cardiovascular risk factors: a randomized controlled trial. Eur J Nutr 57:499. https://doi.org/10.1007/s00394-016-1333-7
Sacks FM, Ornish D, Rosner B et al (1985) Plasma lipoprotein levels in vegetarians: the effect of ingestion of fats from dairy products. JAMA 254:337. https://doi.org/10.1001/jama.1985.03360100087019

Sadrzadeh-Yeganeh H, Elmadfa I, Djazayery A et al (2010) The effects of probiotic and conventional yoghurt on lipid profile in women. Br J Nutr 103:1778. https://doi.org/10.1017/S0007114509993801

Saltzman ET, Palacios T, Thomsen M, Vitetta L (2018) Intestinal microbiome shifts, dysbiosis, inflammation, and non-alcoholic fatty liver disease. Front Microbiol 9:61

Sánchez B, Urdaci MC, Margolles A (2010) Extracellular proteins secreted by probiotic bacteria as mediators of effects that promote mucosa-bacteria interactions. Microbiology 156:3232

Sanchez M, Darimont C, Drapeau V et al (2014) Effect of *Lactobacillus rhamnosus* CGMCC1.3724 supplementation on weight loss and maintenance in obese men and women. Br J Nutr 111:1507. https://doi.org/10.1017/S0007114513003875

Sanders ME (2008) Probiotics: definition, sources, selection, and uses. Clin Infect Dis 46:S58

Sartor RB (2008) Microbial influences in inflammatory bowel diseases. Gastroenterology 134:577. https://doi.org/10.1053/j.gastro.2007.11.059

Sattar N, Preiss D, Murray HM et al (2010) Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials. Lancet 375:735. https://doi.org/10.1016/S0140-6736(09)61965-6

Schmidt KL, Cohn JF (2001) Human facial expressions as adaptations: evolutionary questions in facial expression research. Yearb Phys Anthropol 33:3. https://doi.org/10.1002/ajpa.20001

Serino M, Fernandez-Real JM, Garcia-Fuentes E et al (2013) The gut microbiota profile is associated with insulin action in humans.[Erratum appears in Acta Diabetol. 2013 Oct;50(5): 763 Note: Fuentes, Eduardo Garcia [corrected to Garcia-Fuentes, Eduardo]]. Acta Diabetol. https://doi.org/10.1007/s00592-012-0410-5

Shimazu T, Kuriyama S, Hozawa A et al (2007) Dietary patterns and cardiovascular disease mortality in Japan: a prospective cohort study. Int J Epidemiol 36:600. https://doi.org/10.1093/ije/dym005

Sima P, Vannucci L, Vetvicka V (2018) β-glucans and cholesterol (review). Int J Mol Med 41:1799

Siró I, Kápolna E, Kápolna B, Lugasi A (2008) Functional food. Product development, marketing and consumer acceptance-a review. Appetite 51:456

Slavin J (2013) Fiber and prebiotics: mechanisms and health benefits. Nutrients 22:1417

Slomko H, Heo HJ, Einstein FH (2012) Minireview: epigenetics of obesity and diabetes in humans. Endocrinology 153:1025–1030

Sousa e Silva JP, Freitas AC (2014) Probiotic bacteria: fundamentals, therapy and technological aspects. In: Probiotic bacteria: fundamentals, therapy and technological aspects, pp 1–305

Sun Z, Sun X, Li J et al (2020) Using probiotics for type 2 diabetes mellitus intervention: advances, questions, and potential. Crit Rev Food Sci Nutr 60:670

Tang WHW, Wang Z, Levison BS et al (2013) Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med 368:1575. https://doi.org/10.1056/NEJMoa1109400

Tang WHW, Kitai T, Hazen SL (2017) Gut microbiota in cardiovascular health and disease. Circ Res 120:1183

Terpou A, Papadaki A, Lappa IK et al (2019) Probiotics in food systems: significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. Nutrients 11:1591. https://doi.org/10.3390/nu11071591

Theuwissen E, Mensink RP (2008) Water-soluble dietary fibers and cardiovascular disease. Physiol Behav 94:285

Tolhurst G, Heffron H, Lam YS et al (2012) Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. Diabetes 61:364. https://doi.org/10.2337/db11-1019

Tsai YL, Lin TL, Chang CJ et al (2019) Probiotics, prebiotics and amelioration of diseases. J Biomed Sci 26:3

Turnbaugh PJ, Bäckhed F, Fulton L, Gordon JI (2008) Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell Host Microbe 3:213. https://doi.org/10.1016/j.chom.2008.02.015

Turnbaugh PJ, Hamady M, Yatsunenko T et al (2009) A core gut microbiome in obese and lean twins. Nature 457:480. https://doi.org/10.1038/nature07540

Valenlia KB, Morshedi M, Saghafi-Asl M et al (2018) Beneficial impacts of Lactobacillus plantarum and inulin on hypothalamic levels of insulin, leptin, and oxidative markers in diabetic rats. J Funct Foods. https://doi.org/10.1016/j.jff.2018.04.069

Valls J, Pasamontes N, Pantaleón A et al (2013) Prospects of functional foods/nutraceuticals and markets. In: Natural products: phytochemistry, botany and metabolism of alkaloids, phenolics and terpenes, pp 2491–2525

Vandenplas Y, De Greef E, Devreker T et al (2013) Probiotics and prebiotics in infants and children. Curr Infect Dis Rep 15:251. https://doi.org/10.1007/s11908-013-0334-4

Vaughan EE, Mollet B (1999) Probiotics in the new millennium. Nahrung 43:148

Venturi A, Gionchetti P, Rizzello F et al (1999) Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. Aliment Pharmacol Ther 13:1103. https://doi.org/10.1046/j.1365-2036.1999.00560.x

de la Visitación N, Robles-Vera I, Toral M, Duarte J (2019) Protective effects of probiotic consumption in cardiovascular disease in systemic lupus erythematosus. Nutrients 11:2676

Walker WA (2000) Role of nutrients and bacterial colonization in the development of intestinal host defense. J Pediatr Gastroenterol Nutr 30:S2

Walker WA (2008) Mechanisms of action of probiotics. Clin Infect Dis 46:S87

Wang Z, Klipfell E, Bennett BJ et al (2011) Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 472:57. https://doi.org/10.1038/nature09922

Wang M, Wichienchot S, He X et al (2019) In vitro colonic fermentation of dietary fibers: fermentation rate, short-chain fatty acid production and changes in microbiota. Trends Food Sci Technol 88:1–9. https://doi.org/10.1016/j.tifs.2019.03.005

Weisberg SP, McCann D, Desai M et al (2003) Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112:1796. https://doi.org/10.1172/JCI200319246

Wells JM, Rossi O, Meijerink M, van Baarlen P (2010) Microbes and Health Sackler Colloquium: epithelial crosstalk at the microbiota-mucosal interface. Proc Natl Acad Sci U S A 108:4607. https://doi.org/10.1073/pnas.1000092107

Wong JMW, Esfahani A, Singh N et al (2012) Gut microbiota, diet, and heart disease. J AOAC Int 95:24

Wu X, Ma C, Han L et al (2010) Molecular characterisation of the faecal microbiota in patients with type II diabetes. Curr Microbiol 61:69. https://doi.org/10.1007/s00284-010-9582-9

Xu J, Mahowald MA, Ley RE et al (2007) Evolution of symbiotic bacteria in the distal human intestine. PLoS Biol 5:e156. https://doi.org/10.1371/journal.pbio.0050156

Yadav H, Jain S, Sinha PR (2007) Antidiabetic effect of probiotic dahi containing *Lactobacillus acidophilus* and *Lactobacillus casei* in high fructose fed rats. Nutrition 23:62. https://doi.org/10.1016/j.nut.2006.09.002

Yadav H, Jain S, Sinha PR (2008) Oral administration of dahi containing probiotic *Lactobacillus acidophilus* and *Lactobacillus casei* delayed the progression of streptozotocin-induced diabetes in rats. J Dairy Res 75:189. https://doi.org/10.1017/S0022029908003129

Yadav H, Lee JH, Lloyd J et al (2013) Beneficial metabolic effects of a probiotic via butyrate-induced GLP-1 hormone secretion. J Biol Chem 288:25088. https://doi.org/10.1074/jbc.M113.452516

Yoo JY, Kim SS (2016) Probiotics and prebiotics: present status and future perspectives on metabolic disorders. Nutrients 8:173

Zaura E, Keijser BJ, Huse SM, Crielaard W (2009) Defining the healthy "core microbiome" of oral microbial communities. BMC Microbiol 9:259. https://doi.org/10.1186/1471-2180-9-259

Zaura E, Nicu EA, Krom BP, Keijser BJF (2014) Acquiring and maintaining a normal oral microbiome: current perspective. Front Cell Infect Microbiol 4:85

Zeyda M, Stulnig TM (2007) Adipose tissue macrophages. Immunol Lett 112:61

Zhang X, Shen D, Fang Z et al (2013) Human gut microbiota changes reveal the progression of glucose intolerance. PLoS One 8:e71108. https://doi.org/10.1371/journal.pone.0071108

Zhang C, Ma S, Wu J et al (2020) A specific gut microbiota and metabolomic profiles shifts related to antidiabetic action: the similar and complementary antidiabetic properties of type 3 resistant starch from *Canna edulis* and metformin. Pharmacol Res 159:104985. https://doi.org/10.1016/j.phrs.2020.104985

Zhao L, Shen J (2010) Whole-body systems approaches for gut microbiota-targeted, preventive healthcare. J Biotechnol 149:183. https://doi.org/10.1016/j.jbiotec.2010.02.008

Zhao D, Liu J, Wang M et al (2019) Epidemiology of cardiovascular disease in China: current features and implications. Nat Rev Cardiol 16:203

Zhi C, Huang J, Wang J et al (2019) Connection between gut microbiome and the development of obesity. Eur J Clin Microbiol Infect Dis 38:1987–1998

Implications of Probiotics and Prebiotics on Immune Functions

10

Kavita Pandey and Anam Modi

Abstract

Keeping the digestive system healthy is a foundation stone to general health and well-being. More than 75% of our immunity lies in the gut. The microbes that we harbor in and on our body (the microbiota) is crucial for the GIT functioning and thus immunity. An optimal balance between the good and bad bacteria is the key to good health and stronger immunity. Various lifestyle patterns like incorrect food habits, junk food, faulty sleep patterns, and fast life create imbalance in microbiota causing dysbiosis. Dysbiosis is the root cause of several diseases including hypertension, diabetes, cancer, cardiovascular issues, etc. Incorporating functional foods like probiotics, prebiotics, and their combination (synbiotics) in daily diet helps in selective proliferation of the good bacteria in the gut, and this helps in overcoming the dysbiosis. Probiotics have been assessed for multiple properties like cholesterol-lowering effects and anti-carcinogenic activity. Probiotics exhibit immunomodulatory effects on the hosts. This chapter discusses the effects of probiotic and prebiotic consumption on the immune homeostasis function. Mechanisms of probiosis and prebiosis have been elaborated upon. Although there are numerous studies described in the scientific literature regarding the beneficial effects obtained from the consumption of probiotics, some cases of side effects also prevail which have been taken account of in this chapter.

Keywords

Probiotics · Prebiotics · Functional foods · Microbiota · Immunomodulation

K. Pandey (✉) · A. Modi
Department of Bioanalytical Sciences (FYIC-Integrated), GNIRD, G.N. Khalsa College (Autonomous), Mumbai, India
e-mail: kavita.pandey@gnkhalsa.edu.in

K. K. Behera et al. (eds.), *Prebiotics, Probiotics and Nutraceuticals*,
https://doi.org/10.1007/978-981-16-8990-1_10

10.1 Human Gastrointestinal Tract

The primary function of the human gastrointestinal tract (GIT) was earlier thought to be limited to digestion and absorption of nutrients and excretion of waste end products. However, clinical studies in the last three decades have proved that the GIT fulfils many other functions, which are essential for our well-being; hence it has drawn a greater attention for newly demonstrated applications and effects, especially in immune homeostasis (Wu and Wu 2012; Faith et al. 2013). In an adult human, about 10^{13} microorganisms inhabit the system (the "microbiota"), comprising of more than 500 species and nearly 2 million genes (the "microbiome") (Thursby and Juge 2017). Microbiota is metabolically active and allows synthesis and breakdown of numerous dietary compounds, and thus the microbiota has earned its due significance in our diet. Colonization of the gut with microflora begins even before birth, from the placenta. Feeding pattern seems to modulate the colonization pattern (Gensollen et al. 2016). An optimal "balance" in the microbial population, in terms of good and bad microbes, is essential for the sustenance as well as maintenance of good health in humans (Jain 2020). The effectivity of the immune system is always a reflection of gut functioning, and thus it's imperative to understand the relation between the two.

10.2 GIT and the Immune Regulation

An efficient and healthy immune system is crucial for general health and well-being of an individual. About 80% of our immunity lies in our gut. The immune system is divided into two types—innate (the one we are born with) and acquired (the one we develop after exposure to external agents like microbes or chemicals). The crosstalk between innate and adaptive immune cells and the intestinal microbiota control the equilibrium between immune tolerance and inflammation (Yahfoufi et al. 2018).

Immunoglobulins produced by the B cells are responsible for countering the antigens produced as a result of invasions by foreign bodies like a virus/any pathogen or an allergen. Among the five types of antibodies (IgA, IgD, IgE, IgG, and IgM), IgA acts as the first line of defense and prevents bacterial and viral adhesion to epithelial cells, especially at mucosal sites, saliva, tears, sweat, and breast milk. T cells are the immune cells found in the small and large intestine. The cytotoxic T cells, helper T cells, and memory T cells have established functions in both innate and acquired immunities through cytokine production. Th17 stimulates the gut epithelium to produce antimicrobial peptides (Atarashi et al. 2011). In the course of evolution, the immune cells can recognize the potential pathogens and neutralize them. However they are still largely dependent on the diverse commensals in the gut for functioning. It is the microbiota that educates the immune cells for the defined functions they perform and thus shapes our complete immune system and regulates its responses.

Wu and Wu (2012) have reviewed in depth the mechanisms using which microbiota can modulate our immune functions. This immune system is composed of gut-associated lymphoid tissue (GALT) and other cells, meant to protect the gut from several types of antigens readily supplied from foods, commensal, and pathogenic bacteria (Ashaolu 2020). A dysregulation of immune functions can have deteriorating effects on the general health. Immune system is vulnerable to conditions associated with food and gut functions like malnutrition, junk foods, poor lifestyle, and many a times physical and mental stress. Deficiency of proteins or certain vitamins and mineral also can result in attenuation of the immune system. Right food thus is crucial for good immune functioning and thus physical and mental well-being (Ashaolu 2020).

For instance, vitamin A is known to regulate the Th1/Th2 balance, while vitamin C relieves the oxidative stress, and vitamin E has proven anti-inflammatory properties. Minerals like zinc and selenium are crucial for cell-mediated responses. Peptidoglycans and lipoteichoic acids derived from probiotics stimulate the innate immunity by producing interleukins (specifically IL-12 and IL 10) (Kaminogawa and Nanno 2004).

10.3 Functional Foods

The concept of functional food considers food beyond the necessity for survival, as a source of mental and physical well-being, contributing to the prevention and reduction of risk factors for several diseases or enhancing certain physiological functions (Roberfroid 2000; Cencic and Chingwaru 2010; Wildman 2016). Functional foods contain significant levels of biologically active components like polyunsaturated fatty acids (PUFAs), essential oils, ß-glucans, etc. that provide health benefits much more than basic nutrition.

The use of microbes or microbial products originated (unintentionally) centuries ago when people first noted the beneficial health effects of eating fermented foods. The use of yogurt and fermented milk products is known to mankind since humans and animals started evolving (Hui and Evranuz 2012). The possibility that ingestion of some selected bacteria may beneficially influence the GIT of humans was proposed by Eli Metchnikoff, the Russian Nobel Prize winner (1907) (Guarner et al. 2008). Since then the quest for discovering how food can enhance health or prevent chronic diseases has triggered the research to study a wide range of components in foods, besides nutrients, and this has opened up a new arena called functional foods (Lopez-Varela et al. 2002; Kaminogawa and Nanno 2004; Webb and Webb 2006). We need good bacteria in the gut to elicit positive effects on the host including immunomodulatory benefits (Roberfroid 2000). This can be achieved by supplementation with probiotics, prebiotics, or combination of the two synbiotics, which fall under the canopy of functional foods (Hasler 1998; Shah 2001; Vanhoutte et al. 2006; Methé et al. 2012; Pandey et al. 2015).

10.4 Immune Regulation and Probiotics

Food-derived bioactives are known to have regulatory roles in both types of immunities, and hence good food is the secret to stronger immunity. As per the latest definition put forward jointly by FAO/WHO, Probiotics are "live microorganisms which when administered in adequate amount confer a health benefit on the host" (WHO/FDA 2006). Some of the most commonly used probiotic microorganisms in food and medicines belong to *Lactobacillus* and *Bifidobacterium* genera. More advanced and focused research efforts have led to emergence of new genera and probiotic strains. Probiotic products are available as single strain (e.g., *Bacillus coagulans* GBI-30 6086) or a mixture of two or more strains (Yakult). Health benefits imparted by probiotics are strain specific and cannot be generalized, as their activities may differ when used singly and when combined with other strains. Examples of probiotic products in market include Nesvita, probiotic lassi, probiotic dahi and ice cream, probiotic yogurts, probiotic chocolates, probiotic bakery products, etc. Ideal characteristics of a probiotic organism have been discussed by Pandey et al. (2015).

10.4.1 Mechanisms of Probiosis

General mechanisms of probiotics have been discussed by many researchers (Hemaiswarya et al. 2013; Pandey et al. 2015; Khare et al. 2018). GALT is responsible for eliciting the immune responses by enhanced cytokine production and associated roles (Patel and Goyal 2012). Toward immune homeostasis, probiotics primarily works through modulation of mucosal immunity and gut barrier defense (Hardy et al. 2013). They are capable of regulating the activity of immune cells like natural killer cells, dendritic cells, macrophages, granulocytes, T helper cells (Th1/Th2/Th17), B cells, etc. Probiotics have shown to exert beneficial effects in immunopathological conditions like IBD and hypersensitivity, which are reflection of their potential as immune regulators including activation, suppression, regulation, etc. (Ezendam and van Loveren 2006; Azad et al. 2018).

As a part of regular metabolism, probiotics produce various metabolites like SCFA (short chain fatty acids), bacteriocins, etc. (Mathipa and Thantsha 2017). IL production and NK cell activity also are modulated by these SCFAs (Hijova and Chmelarova 2007; Jakobsdottir et al. 2013; Morrison and Preston 2016; Khare et al. 2018). The most abundant SCFA in colon is acetate, which can also be utilized by some butyrate-producing bacteria in the gut. SCFAs not only reduce the pH in colon but also suppress inflammation and promote excretion of ammonia and amine (Morrison and Preston 2016). SCFAs enhance the antimicrobial peptide production by epithelial cells, which in turn modulate the cytokine and chemokine regulation leading to activation of immune cells and differentiation of T-lymphocytes. Furthermore, SCFAs promote proliferation of other cytokines like IL-18, known for roles in maintenance and repair of epithelial integrity (McKeen et al. 2019). Paneth cells (crypts of small intestine), intestinal epithelial cells, and other cell types in probiotic strains are known to produce antimicrobial peptides named defensins (Sang and Blecha 2008). They stimulate defensin production by producing proteases or MMPs. Nuclear factor (NF)-κB coordinates all the immune and inflammatory responses against pathogens and other stress signals (O'Hara and Shanahan 2007).

Saulnier and his coworkers strongly advocate the significance of surface proteins of probiotic cells in the immunomodulation functions (Saulnier et al. 2009). The probiotics are able to stimulate the immune system by enhancing the productions of mucosal antibody or pro-inflammatory cytokines or host defensins. They suppress the immunity by lowering the cytokine production and cellular proliferation coupled with increased apoptosis.

Intake of yogurt (containing 2.5×10^{10} cells of *L. delbruecki* subsp. bulgaricus OLL1073R-1) by elderly population lowered their risk of catching common cold by a factor of 2.6 (Makino et al. 2010). In another study, ingestion of a probiotic drink containing *Lactobacillus casei Shirota* (1.3×0^{10} live cells) was associated with enhanced expression of CD69 activation marker (which is a negative regulator of inflammatory responses), parallel inducing secretion of mucosal salivary IgA1 and IgA2 concentrations. Furthermore, a drop in cellular pro-inflammatory cytokine profile was observed (Dong et al. 2010).

Team led by Van den Nieuwboer conducted 57 intervention studies to evaluate the safety of using probiotics and synbiotics in immune compromised adults (Van den Nieuwboer et al. 2015). No side effects were observed; hence the formulation was safe. In another study, by Kim et al. (2016), potential of probiotics, as safe and acceptable therapeutic intervention to enhance gut immune restoration in HIV patients, was evaluated and found to be encouraging.

A study was conducted by Horvath et al. (2016) with liver cirrhosis patients, to establish the role of multispecies probiotics on their immunity and gut permeability. Increased neopterin levels and more number of ROS released by neutrophils support the claim of beneficial effect of probiotics on immune functioning. NK cell activity was affected. Furthermore, supplementation of the probiotic strains may improve liver function. On the contrary, the gut barrier function is minimal.

A systematic review, to establish the immune-related effects of supplementation of *Bifidobacterium animalis* ssp. lactis HN019, in healthy elderly subjects was undertaken by Miller et al. (2017). The study concluded that daily supplementation of *B. lactis* HN019 (5×10^{10} to 3×10^{11} CFU/day) improved the NK cell and PMN function in healthy elderly adults. However, the study was limited to smaller groups, and the effects need to be warranted with study in larger groups. Du et al. (2018) evaluated the effects of supplementing piglets with probiotic *Bacillus amyloliquefaciens* SC06 on the gut epithelial barrier and immune functions. Ninety males were divided into three groups. Diet of groups 1, 2, and 3 were supplemented with antibiotic Aureomycin (150 mg/kg), combination of Aureomycin (75 mg/kg) and probiotic (1×10^{8} CFU/kg), and only probiotic (2×10^{8} CFU/kg) respectively. The probiotic supplementation significantly increased tumor necrosis factor (TNF)-α and IL-6 secretions and decreased serum interferon (IFN)-α, IFN-γ, interleukin (IL)-1β, and IL-4 levels. The diet was also associated with downregulation of TNF and IL-1α expressions in intestinal mucosa and upregulation of IL-6 and IL-8 transcription, thus demonstrating the enhanced gut barrier function. Additionally, the supplementation also activated the TLR signaling pathway. Thus, incorporating *Bacillus amyloliquefaciens* SC06 in diet could be a potential alternative to use of antibiotics.

Martens et al. (2018) have reviewed the effects of probiotic supplementations in the prevention and/or treatment of chronic airway diseases. The probiotic cells are known to interact with the immune cells and stimulate or suppress the responses, end result of which is restoration of defective epithelial barrier. A 4-week intervention study with supplementation of combination of *Lactobacillus rhamnosus* PB01 DSM14869 and *Lactobacillus curvatus* EB10 DSM32307 demonstrated positive effect on the gingival health. Interleukins analyzed from subjects included IL-1β, IL-10, IL-8, IL-6, and TNF-α. No marked immune response was noted when compared to placebo group (Keller et al. 2018). There also exist some studies where no significant benefit was correlated to the probiotic supplementation (Braathen et al. 2017; Ibrahim et al. 2017).

It is noteworthy that the microbiota does not interact with the epithelial cells directly, but the metabolites they produce stimulate the maturation and functional properties of the immune cells. This was further ascertained by a study from Galdeano and team (Galdeano et al. 2019), who demonstrated the enhanced adhesion of *L. casei and L. paracasei* to the intestinal epithelial cells through TLRs and stimulate the immune system.

10.4.2 Debated Roles of Probiotics

Probiotics are safe for most of the population, but side effects can occur. The most common side effects include excessive gas formation and bloating. Probiotic-rich foods are known to contain amines (like histamine, tyramine, tryptamine, and phenylethylamine, which have been linked with headaches) Probiotics like *Lactobacillus buchneri* and *Lactobacillus helveticus* are histamine producers, which cause symptoms similar to allergic reactions in several patients. In more serious and rare instances, probiotics can cause infections, especially in immune-compromised people (Didari et al. 2014; Szajewska et al. 2014).

10.5 Immune Regulation and Prebiotics

Prebiotics are essentially food for probiotics (and potentially for other intestinal bacteria too). Initially, prebiotics concept was limited to complex carbohydrate ingredients; however with deeper insights into the crosstalks between prebiotic substrates and the gut microbiota, the term prebiotics has been defined and redefined multiple times (Pandey et al. 2015; Davani-Davari et al. 2019). ISAPP in June 2017 defined it as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Gibson et al. 2017). Some of the sources of prebiotics include breast milk, soybeans, and inulin sources (like Jerusalem artichoke, chicory roots, etc.), raw oats, unrefined wheat, unrefined barley, yacón, non-digestible carbohydrates, and in particular non-digestible oligosaccharides. Prebiotics like inulin and pectin exhibit several health benefits like reducing the prevalence and duration of diarrhea, relief from inflammation, and other symptoms associated with intestinal bowel disorder and protective effects to prevent colon cancer (Peña 2007; Al-Sheraji et al. 2013; Song et al. 2014; Pandey et al. 2015). Immune system get affected due to change in intestinal microflora, which in turn get influenced by prebiotics (Schley and Field 2002; Shokryazdan et al. 2017).

10.6 Mechanisms of Prebiotics

One of the prerequisites of prebiotic effect is the target microbes present in the gut which possesses specific enzymes capable of catalysis breakdown of prebiotics. Complex pathways exist which result in array of metabolites, which results in a cross-feeding and is the key to prebiotic functional attributes. These compounds may directly stimulate immunity, protect against pathogens, and facilitate host metabolism (Swennen et al. 2006; Shoaib et al. 2016). By changing the composition and functionality of the microbiota, prebiotics play a role not only by facilitating competitive exclusion of potential pathogens but also in modulating the immune system and enhancing host defense. Additionally, they serve as source of energy for epithelial cells and have control over the inflammation modulators and metabolic regulators. Bermudez-Brito et al. (2012) have reviewed and discussed in detail the major mechanisms of action, which include enhancement of the epithelial barrier, increased adhesion to intestinal mucosa, concomitant inhibition of pathogen adhesion, competitive exclusion of pathogenic microorganisms, production of anti-microorganism substances, and modulation of the immune system. Short-chain fatty acids (SCFAs) are important end products formed during colonic fermentation of dietary fiber. SCFAs are absorbed effectively in the cecum with only 5–10% being excreted in the feces (Hijova and Chmelarova 2007). The most abundant SCFA in colon is acetate, which can also be utilized by some butyrate-producing bacteria in the gut. SCFAs not only reduce the pH in colon but also suppress inflammation and promote excretion of ammonia and amine (Morrison and Preston 2016). FOS increases the SCFA production, majorly acetate propionate and butyrate. Lactate-utilizing bacteria have the ability to produce butyrate. FOS are also able to increase the secretion of peptides by the gastrointestinal diffuse neuroendocrine system via SCFA production. SCFAs are also known to decrease the cellular proliferation and induce apoptosis (more effectively the colon tumor cells). IL production and NK cell activity also are modulated by these SCFAs by one of the mechanisms (Jakobsdottir et al. 2013; Morrison and Preston 2016).

10.6.1 Clinical Studies Supporting the Immunomodulatory Benefits of Prebiotics

Blend of prebiotics—oligofructose and inulin—has shown to improve antibody response against influenza virus and others (Dong et al. 2010). Upregulation of interleukin 4 (IL-4) levels in serum, CD282+/TLR2+ myeloid dendritic cells, and a toll-like receptor 2-mediated immuneresponse in healthy volunteers was demonstrated on feeding β-fructans (O'Flaherty et al. 2010). Supplementation of acidic oligosaccharides reduced the risk of atopic dermatitis and eczema in low-risk infants (Donkor et al. 2012).

Recently, a study reported increase in four microbial species on supplementation with fibruline and arabinoxylooligosaccharides in different donors (i.e., *Bacteroides*, *Bifidobacterium*, *Erysipelotrichaceae*, and *Prevotella*). This increase in specific microbial communities was further linked to the gut barrier functions and immunomodulation with regulation of IL-6 and IL-10 (Van den Abbeele et al. 2018). Effect of dietary galactooligosaccharide (0, 1, and 2%) was studied in narrow 216 clawed crayfishes by Nedaei et al. (2019). The group proposes that 2% GOS supplementation increased proliferation rates of hemocytes, and this may induce improved immune functions against environmental stress and opportunistic pathogens. For continuous benefits, the supplementation should not be stopped. The study also confirmed the effect of GOS supplementation on enhanced innate immunity.

Findings of Perdijk et al. (2019) demonstrate the positive effects of human milk oligosaccharide-sialyllactose and GOS on the epithelial barrier functioning using in vitro models by studying the re-epithelialization, proliferation, and differentiation of the chosen cell line—CaCo-2. They selectively promoted the growth of *Lactobacillus* and *Bifidobacterium* in the gut. Additionally, the SCFA exerted systemic responses and served anti-inflammatory functions by suppressing colitis. Another promising health benefit is the possible prevention or delay in the onset of diabetes mellitus (Ivison et al. 2010). Some of the studies reported that the prebiotic showed no beneficial effect on human immune system. A study undertaken by Stefaniak et al. (2019) evaluated the potential of early in ovo administration of prebiotics (inulin and Bi2tos – galacto-oligosaccharide) in development of innate immune system in broilers. The results demonstrated the stimulatory effect of prebiotics consumption on hematopoiesis.

10.6.2 Debated Roles of Prebiotics

Overdosing of prebiotics, in some cases, may cause discomfort, but is not life-threatening. Fermentation of FOS in the colon leads to production of hydrogen and carbon dioxide which can cause discomfort to people. Excessive intake of prebiotics especially oligosaccharides like FOS, GOS, etc. causes abdominal uneasiness like bloating and distension, as well as significant levels of flatulence. Some prebiotics like inulin-derived oligosaccharides have mild laxative effects (Louis

Table 10.1 Clinical data supporting use of probiotics/prebiotics for immunomodulatory benefits

Probiotic/prebiotics/ synbiotics	Experimental design	Key metabolites involved	Ref
B. animalis subsp. lactis Bl-04 2	150 days	WBC, neutrophil, monocyte, lymphocyte, eosinophil	West et al. (2014)
L. acidophilus NCFM and *B. animalis* subsp. lactis BI-07	One sachet daily of either 2.0×10^9 CFU per day of *B. animalis* subsp. lactis Bl-04 (Bl-04; Danisco USA, Madison, WI) or *L. acidophilus* NCFM and *B. animalis* subsp. lactis Bi-07 (Danisco USA) 1.0×10^{10} CFU per day (5.0×10^9 CFU of each strain) in a 1 g sucrose base or placebo powder, 125 healthy humans		
Heat-killed *L. gasseri* (TMC0356)	Human trial with 4 weeks supplementation of 28 elderly subjects (50–70 years), with 2 g of probiotics	WBC, neutrophil, monocyte, lymphocyte, eosinophil, CD4, CD8, NK cell count, NK activity was observed	Miyazawa et al. (2015)
Bacillus coagulans GBI-30	4 weeks Each capsule of BC30 contained 1.3×10^9 CFUs, 36 older men and women	NK cell activity	Nyangale et al. (2015)
L. paracasei K71	3 weeks, 20 healthy humans	WBC	Saito et al. (2017)
L. casei strain Shirota	8 weeks; 47 healthy medical students	NK cell count and NK activity	Kato-Kataoka et al. (2016)
L. plantarum CECT 7315 and 7316 in 20 g of powdered skimmed milk	12 weeks 3.6×10^7 CFU/mL, 205 humans	CD4, CD8, B cell, T cell	Pu et al. (2017)
L. johnsonii N6.2	8 weeks, 42 healthy elderly humans	Monocyte, CD4, CD8, NK cell count	Marcial et al. (2017)
B. bifidum, *B. breve*, *B. longum*	12 months, 48 infants	Composition and function of microbiota of infants in the first year of life	Bazanella et al. (2017)
L. acidophilus and FOS	8 weeks (synbiotic group—individuals who consumed 40 g/day of SDM; *n* = 23) and group P (placebo group—	Reduced total cholesterol and immunoglobulins (A and M) and interleukin-1β	Xavier-Santos et al. (2018)

(continued)

Table 10.1 (continued)

Probiotic/prebiotics/ synbiotics	Experimental design	Key metabolites involved	Ref
	individuals who consumed 40 g/day of PDM; $n = 22$); 45 human adults		
Grapeseed flour enriched with prebiotic polyphenols and kefir-derived lactic acid bacteria	9 weeks, propionic acid, animal (ten mice)	Adipogenesis, permeable intestine, systematic inflammation, glucose, and insulin resistance	Cho et al. (2018)
Aronia juice enriched with prebiotic polyphenols	In vitro Caco-2 and endothelial cell co-culture were used, increased propionate, butyrate, *Akkermansia*, and *Firmicutes*, 2 weeks	Pro-inflammatory markers such as interleukin-8 and monocytes were downregulated	Wu et al. (2018)
Prebiotic (hesperidin)	4 weeks, lactobacilli, enterococci, staphylococci, animal (18 rats)	Immune modulation and upregulation of immunoglobulin A and lymphocytes	Estruel-Amades et al. (2019)
Prebiotic (chicory-derived inulin)	2-week run-in 2-week intervention 2-week washout crossover, Human (44 healthy adults)	Bowel function, stool consistency, constipation	
Mixture of GOS and polydextrose	24 and 7 g/L of polydextrose and GOS, male piglets	They may have neurodevelopment effect in human infants	Mudd et al. (2016)
FOS	28 days 3 × 5g/day, healthy volunteers	34. IL-4 in serum, CD282+/TLR2+ myeloid dendritic cells, and toll-like receptor 2-mediated immune response were upregulated	Clarke et al. (2016)
Galacto-oligosaccharides	10-week intervention 4-week washout crossover, Human (40 healthy elderly)	Age-associated alterations in microbiota, improvements in immune markers IL-8, IL-10, and IL-1 beta	Vulevic et al. (2015)
Dietary fiber (soluble corn fiber)	4 weeks 10 and 20 g/day, human (28 females)	Increased *Parabacteroides*, *Bifidobacterium*, *Dialister* Decreased *Anaerostipes*, *Dorea*, *Ruminococcus* Decreased fecal pH, numeric increase in SCFA	Whisner et al. (2016)

et al. 2016). Thus a better understanding of interactions between prebiotics and probiotics is needed (Table 10.1).

10.7 Conclusions

Probiotics have several effects on the host's health metabolism and immune functions. Deeper understanding of the immune homeostasis mechanisms of probiotics will allow us to design enhanced functional foods for improved health. The ability of probiotics to alter the microflora can be used in developing lines of treatment for gastrointestinal disorders. Furthermore, the health claims made need to be authenticated and validated by designing large-scale clinical trials. Also, the growing body of evidence for the immunomodulatory benefits of prebiotics (alone as well as in formulation with probiotic strains, as synbiotic) represents a realistic approach in disease modification. Rapid developments in the sequencing technologies coupled with metabolic profiling techniques, combined with mathematical integration approaches, promise plethora of applications with far-reaching implications.

References

Al-Sheraji SH, Ismail A, Manap MY, Mustafa S, Yusof RM, Hassan FA (2013) Prebiotics as functional foods: a review. J Funct Foods 5(4):1542–1553

Ashaolu TJ (2020) Immune boosting functional foods and their mechanisms: a critical evaluation of probiotics and prebiotics. Biomed Pharmacother 130:110625

Atarashi K, Umesaki Y, Honda K (2011) Microbiotal influence on T cell subset development. Semin Immunol 23(2):146–153

Azad M, Kalam A, Sarker M, Wan D (2018) Immunomodulatory effects of probiotics on cytokines profiles. BioMed Res Int. 2018;8063647. doi: https://doi.org/10.1155/2018/8063647

Bazanella M, Maier TV, Clavel T, Lagkouvardos I, Lucio M, Maldonado-Gòmez MX, Autran C, Walter J, Bode L, Schmitt-Kopplin P, Haller D (2017) Randomized controlled trial on the impact of early-life intervention with bifidobacteria on the healthy infant fecal microbiota and metabolome. Am J Clin Nutr 106(5):1274–1286

Bermudez-Brito M, Plaza-Díaz J, Muñoz-Quezada S, Gómez-Llorente C, Gil A (2012) Probiotic mechanisms of action. Ann Nutr Metab 61(2):160–174

Braathen G, Ingildsen V, Twetman S, Ericson D, Jørgensen MR (2017) Presence of Lactobacillus reuteri in saliva coincide with higher salivary IgA in young adults after intake of probiotic lozenges. Benef Microbes 8(1):17–22

Cencic A, Chingwaru W (2010) The role of functional foods, nutraceuticals, and food supplements in intestinal health. Nutrients 2(6):611–625

Cho YJ, Lee HG, Seo KH, Yokoyama W, Kim H (2018) Antiobesity effect of prebiotic polyphenol-rich grape seed flour supplemented with probiotic kefir-derived lactic acid bacteria. J Agric Food Chem 66(47):12498–12511

Clarke ST, Green-Johnson JM, Brooks SP, Ramdath DD, Bercik P, Avila C, Inglis GD, Green J, Yanke LJ, Selinger LB, Kalmokoff M (2016) β2-1Fructan 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. Br J Nutr 115(10):1748–1759

Davani-Davari D, Negahdaripour M, Karimzadeh I, Seifan M, Mohkam M, Masoumi SJ, Berenjian A, Ghasemi Y (2019) Prebiotics: definition, types, sources, mechanisms, and clinical applications. Foods 8(3):92
Didari T, Solki S, Mozaffari S, Nikfar S, Abdollahi M (2014) A systematic review of the safety of probiotics. Expert Opin Drug Saf 13(2):227–239
Dong H, Rowland I, Tuohy KM, Thomas LV, Yaqoob P (2010) Selective effects of Lactobacillus casei Shirota on T cell activation, natural killer cell activity and cytokine production. Clin Exp Immunol 161(2):378–388
Donkor ON, Ravikumar M, Proudfoot O, Day SL, Apostolopoulos V, Paukovics G, Vasiljevic T, Nutt SL, Gill H (2012) Cytokine profile and induction of T helper type 17 and regulatory T cells by human peripheral mononuclear cells after microbial exposure. Clin Exp Immunol 167(2): 282–295
Du W, Xu H, Mei X, Cao X, Gong L, Wu Y, Li Y, Yu D, Liu S, Wang Y, Li W (2018) Probiotic Bacillus enhance the intestinal epithelial cell barrier and immune function of piglets. Benef Microbes 9(5):743–754
Estruel-Amades S, Massot-Cladera M, Pérez-Cano FJ, Franch À, Castell M, Camps-Bossacoma M (2019) Hesperidin effects on gut microbiota and gut-associated lymphoid tissue in healthy rats. Nutrients 11(2):324
Ezendam J, van Loveren H (2006) Probiotics: immunomodulation and evaluation of safety and efficacy. Nutr Rev 64(1):1–14
Faith JJ, Guruge JL, Charbonneau M, Subramanian S, Seedorf H, Goodman AL, Clemente JC, Knight R, Heath AC, Leibel RL, Rosenbaum M (2013) The long-term stability of the human gut microbiota. Science 341(6141):1237439
Galdeano CM, Cazorla SI, Dumit JML, Vélez E, Perdigón G (2019) Beneficial effects of probiotic consumption on the immune system. Ann Nutr Metab 74(2):115–124
Gensollen T, Iyer SS, Kasper DL, Blumberg RS (2016) How colonization by microbiota in early life shapes the immune system. Science 352(6285):539–544
Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ et al (2017) Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol 14(8):491
Guarner F, Khan AG, Garisch J, Eliakim R, Gangl A, Thomson A, Krabshuis J, Le Mair T, Kaufmann P, De Paula JA, Fedorak R (2008) World Gastroenterology Organisation practice guideline: probiotics and prebiotics—May 2008: guideline. South Afri Gastroenterol Rev 6(2): 14–25
Hardy H, Harris J, Lyon E, Beal J, Foey AD (2013) Probiotics, prebiotics and immunomodulation of gut mucosal defences: homeostasis and immunopathology. Nutrients 5(6):1869–1912
Hasler CM (1998) Pre-and probiotics: where are we today? Introduction. Br J Nutr 80(4):S195
Hemaiswarya S, Raja R, Ravikumar R, Carvalho IS (2013) Mechanism of action of probiotics. Braz Arch Biol Technol 56(1):113–119
Hijova E, Chmelarova A (2007) Short chain fatty acids and colonic health. Bratisl Lek List 108(8): 354
Horvath A, Leber B, Schmerboeck B, Tawdrous M, Zettel G, Hartl A, Madl T, Stryeck S, Fuchs D, Lemesch S, Douschan P (2016) Randomised clinical trial: the effects of a multispecies probiotic vs. placebo on innate immune function, bacterial translocation and gut permeability in patients with cirrhosis. Aliment Pharmacol Ther 44(9):926–935
Hui YH, Evranuz EÖ (eds) (2012) Handbook of fermented food and beverage technology two volume set. CRC
Ibrahim NS, Ooi FK, Chen CK, Muhamad AS (2017) Effects of probiotics supplementation and circuit training on immune responses among sedentary young males. J Sports Med Phys Fitness 58(7-8):1102–1109
Ivison SM, Wang C, Himmel ME, Sheridan J, Delano J, Mayer ML, Yao Y, Kifayet A, Steiner TS (2010) Oxidative stress enhances IL-8 and inhibits CCL20 production from intestinal epithelial

cells in response to bacterial flagellin. Am J Physiol Gastrointest Liver Physiol 299(3):G733–G741

Jain S (2020) Probiotics and their significance in therapeutic nutrition. IP J Nutr Metab Health Sci 3(1):13–21

Jakobsdottir G, Blanco N, Xu J, Ahrné S, Molin G, Sterner O, Nyman M (2013) Formation of short-chain fatty acids, excretion of anthocyanins, and microbial diversity in rats fed blackcurrants, blackberries, and raspberries. J Nutr Metab. 2013:202534. doi: https://doi.org/10.1155/2013/202534

Kaminogawa S, Nanno M (2004) Modulation of immune functions by foods. Evid Based Complement Alternat Med 1(3):241–250

Kato-Kataoka A, Nishida K, Takada M, Kawai M, Kikuchi-Hayakawa H, Suda K, Ishikawa H, Gondo Y, Shimizu K, Matsuki T, Kushiro A (2016) Fermented milk containing Lactobacillus casei strain Shirota preserves the diversity of the gut microbiota and relieves abdominal dysfunction in healthy medical students exposed to academic stress. Appl Environ Microbiol 82(12):3649–3658

Keller MK, Brandsborg E, Holmstrøm K, Twetman S (2018) Effect of tablets containing probiotic candidate strains on gingival inflammation and composition of the salivary microbiome: a randomised controlled trial. Benef Microbes 9(3):487–494

Khare A, Thorat G, Bhimte A, Yadav V (2018) Mechanism of action of prebiotic and probiotic. J Entomol Zool Stud 6(4):51–53

Kim CJ, Walmsley SL, Raboud JM, Kovacs C, Coburn B, Rousseau R, Reinhard R, Rosenes R, Kaul R (2016) Can probiotics reduce inflammation and enhance gut immune health in people living with HIV: study designs for the Probiotic Visbiome for Inflammation and Translocation (PROOV IT) pilot trials. HIV Clin Trials 17(4):147–157

Lopez-Varela S, González-Gross M, Marcos A (2002) Functional foods and the immune system: a review. Eur J Clin Nutr 56(3):S29–S33

Louis P, Flint HJ, Michel C (2016) How to manipulate the microbiota: prebiotics. Microbiota of the human body 119-142

Makino S, Ikegami S, Kume A, Horiuchi H, Sasaki H, Orii N (2010) Reducing the risk of infection in the elderly by dietary intake of yoghurt fermented with Lactobacillus delbrueckii ssp. bulgaricus OLL1073R-1. Br J Nutr 104(7):998–1006

Marcial GE, Ford AL, Haller MJ, Gezan SA, Harrison NA, Cai D, Meyer JL, Perry DJ, Atkinson MA, Wasserfall CH, Garrett T (2017) Lactobacillus johnsonii N6. 2 modulates the host immune responses: a double-blind, randomized trial in healthy adults. Front Immunol 8:655

Martens K, Pugin B, De Boeck I, Spacova I, Steelant B, Seys SF, Lebeer S, Hellings PW (2018) Probiotics for the airways: potential to improve epithelial and immune homeostasis. Allergy 73(10):1954–1963

Mathipa MG, Thantsha MS (2017) Probiotic engineering: towards development of robust probiotic strains with enhanced functional properties and for targeted control of enteric pathogens. Gut Pathog 9(1):28

McKeen S, Young W, Mullaney J, Fraser K, McNabb WC, Roy NC (2019) Infant complementary feeding of prebiotics for the microbiome and immunity. Nutrients 11(2):364

Methé BA, Nelson KE, Pop M, Creasy HH, Giglio MG, Huttenhower C, Gevers D, Petrosino JF, Abubucker S, Badger JH, Chinwalla AT (2012) A framework for human microbiome research. Nature 486(7402):215

Miller LE, Lehtoranta L, Lehtinen MJ (2017) The effect of Bifidobacterium animalis ssp. lactis HN019 on cellular immune function in healthy elderly subjects: systematic review and meta-analysis. Nutrients 9(3):191

Miyazawa K, Kawase M, Kubota A, Yoda K, Harata G, Hosoda M, He F (2015) Heat-killed Lactobacillus gasseri can enhance immunity in the elderly in a double-blind, placebo-controlled clinical study. Benef Microbes 6(4):441–449

Morrison DJ, Preston T (2016) Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. Gut Microbes 7(3):189–200

Mudd AT, Alexander LS, Berding K, Waworuntu RV, Berg BM, Donovan SM, Dilger RN (2016) Dietary prebiotics, milk fat globule membrane, and lactoferrin affects structural neurodevelopment in the young piglet. Front Pediatr 4:4

Nedaei S, Noori A, Valipour A, Khanipour AA, Hoseinifar SH (2019) Effects of dietary galactooligosaccharide enriched commercial prebiotic on growth performance, innate immune response, stress resistance, intestinal microbiota and digestive enzyme activity in Narrow clawed crayfish (Astacus leptodactylus Eschscholtz, 1823). Aquaculture 499:80–89

Nyangale EP, Farmer S, Cash HA, Keller D, Chernoff D, Gibson GR (2015) Bacillus coagulans GBI-30, 6086 modulates Faecalibacterium prausnitzii in older men and women. J Nutr 145(7): 1446–1452

O'Flaherty S, Saulnier D, Pot B, Versalovic J (2010) How can probiotics and prebiotics impact mucosal immunity? Gut microbes 1(5):293–300

O'Hara AM, Shanahan F (2007) Mechanisms of action of probiotics in intestinal diseases. ScientificWorldJournal 7:1–46

Pandey KR, Naik SR, Vakil BV (2015) Probiotics, prebiotics and synbiotics—a review. J Food Sci Technol 52(12):7577–7587

Patel S, Goyal A (2012) The current trends and future perspectives of prebiotics research: a review. 3 Biotech 2(2):115–125

Peña AS (2007) Intestinal flora, probiotics, prebiotics, synbiotics and novel foods. Rev Esp Enferm Dig 99(11):653

Perdijk O, Van Baarlen P, Fernandez-Gutierrez MM, Van Den Brink E, Schuren FH, Brugman S, Savelkoul HF, Kleerebezem M, Van Neerven RJ (2019) Sialyllactose and galactooligosaccharides promote epithelial barrier functioning and distinctly modulate microbiota composition and short chain fatty acid production in vitro. Front Immunol 10:94

Pu F, Guo Y, Li M, Zhu H, Wang S, Shen X, He M, Huang C, He F (2017) Yogurt supplemented with probiotics can protect the healthy elderly from respiratory infections: a randomized controlled open-label trial. Clin Interv Aging 12:1223

Roberfroid MB (2000) Prebiotics and probiotics: are they functional foods? Am J Clin Nutr 71(6): 1682S–1687S

Saito Y, Fujii M, Watanabe T, Maruyama K, Kowatari Y, Ogata H, Kumagai T (2017) Randomized, double-blind, placebo-controlled, parallel-group study of the effect of Lactobacillus paracasei K71 intake on salivary release of secretory immunoglobulin A. Biosci Microbiota Food Health 36(2):55–63. https://doi.org/10.12938/bmfh.16-022

Sang Y, Blecha F (2008) Antimicrobial peptides and bacteriocins: alternatives to traditional antibiotics. Anim Health Res Rev 9(2):227–235

Saulnier DM, Spinler JK, Gibson GR, Versalovic J (2009) Mechanisms of probiosis and prebiosis: considerations for enhanced functional foods. Curr Opin Biotechnol 20(2):135–141

Schley PD, Field CJ (2002) The immune-enhancing effects of dietary fibres and prebiotics. Br J Nutr 87(S2):S221–S230

Shah NP (2001) Functional foods from probiotics and prebiotics: functional foods from probiotics and prebiotics. Food Technol 55(11):46–53

Shoaib M, Shehzad A, Omar M, Rakha A, Raza H, Sharif HR, Shakeel A, Ansari A, Niazi S (2016) Inulin: properties, health benefits and food applications. Carbohydr Polym 147:444–454

Shokryazdan P, Jahromi MF, Navidshad B, Liang JB (2017) Effects of prebiotics on immune system and cytokine expression. Med Microbiol Immunol 206(1):1–9

Song SK, Beck BR, Kim D, Park J, Kim J, Kim HD, Ringø E (2014) Prebiotics as immunostimulants in aquaculture: a review. Fish Shellfish Immunol 40(1):40–48

Stefaniak T, Madej JP, Graczyk S, Siwek M, Łukaszewicz E, Kowalczyk A, Sieńczyk M, Bednarczyk M (2019) Selected prebiotics and synbiotics administered in ovo can modify innate immunity in chicken broilers. BMC Vet Res 15(1):105

Swennen K, Courtin CM, Delcour JA (2006) Non-digestible oligosaccharides with prebiotic properties. Crit Rev Food Sci Nutr 46(6):459–471

Szajewska H, Guarino A, Hojsak I, Indrio F, Kolacek S, Shamir R, Vandenplas Y, Weizman Z (2014) Use of probiotics for management of acute gastroenteritis: a position paper by the ESPGHAN Working Group for Probiotics and Prebiotics. J Pediatr Gastroenterol Nutr 58(4): 531–539

Thursby E, Juge N (2017) Introduction to the human gut microbiota. Biochem J 474(11): 1823–1836

Van den Abbeele P, Taminiau B, Pinheiro I, Duysburgh C, Jacobs H, Pijls L, Marzorati M (2018) Arabinoxylo-oligosaccharides and inulin impact inter-individual variation on microbial metabolism and composition, which immunomodulates human cells. J Agric Food Chem 66(5): 1121–1130

Van den Nieuwboer M, Brummer RJ, Guarner F, Morelli L, Cabana M, Claassen E (2015) The administration of probiotics and synbiotics in immune compromised adults: is it safe? Benef Microbes 6(1):3–17

Vanhoutte T, De Preter V, De Brandt E, Verbeke K, Swings J, Huys G (2006) Molecular monitoring of the fecal microbiota of healthy human subjects during administration of lactulose and Saccharomyces boulardii. Appl Environ Microbiol 72(9):5990–5997

Vulevic J, Juric A, Walton GE, Claus SP, Tzortzis G, Toward RE, Gibson GR (2015) Influence of galacto-oligosaccharide mixture (B-GOS) on gut microbiota, immune parameters and metabonomics in elderly persons. Br J Nutr 114(4):586–595

Webb GP, Webb GP (2006) Dietary supplements and functional foods. Blackwell, pp 210–215

West NP, Horn PL, Barrett S, Warren HS, Lehtinen MJ, Koerbin G, Brun M, Pyne DB, Lahtinen SJ, Fricker PA, Cripps AW (2014) Supplementation with a single and double strain probiotic on the innate immune system for respiratory illness. e-SPEN J 9(5):e178–e184

Whisner CM, Martin BR, Nakatsu CH, Story JA, MacDonald-Clarke CJ, McCabe LD, McCabe GP, Weaver CM (2016) Soluble corn fiber increases calcium absorption associated with shifts in the gut microbiome: a randomized dose-response trial in free-living pubertal females. J Nutr 146(7):1298–1306

Wildman RE (ed) (2016) Handbook of nutraceuticals and functional foods. CRC

World Health Organization, Food and Agricultural Organization of the United Nations (2006) Probiotics in food: health and nutritional properties and guidelines for evaluation. Food and Nutrition. World Health Organization, Rome

Wu HJ, Wu E (2012) The role of gut microbiota in immune homeostasis and autoimmunity. Gut Microbes 3(1):4–14

Wu T, Grootaert C, Pitart J, Vidovic NK, Kamiloglu S, Possemiers S, Glibetic M, Smagghe G, Raes K, Van de Wiele T, Van Camp J (2018) Aronia (Aronia melanocarpa) polyphenols modulate the microbial community in a Simulator of the Human Intestinal Microbial Ecosystem (SHIME) and decrease secretion of proinflammatory markers in a Caco-2/endothelial cell coculture model. Mol Nutr Food Res 62(22):1800607

Xavier-Santos D, Lima ED, Simão ANC, Bedani R, Saad SMI (2018) Effect of the consumption of a synbiotic diet mousse containing Lactobacillus acidophilus La-5 by individuals with metabolic syndrome: a randomized controlled trial. J Funct Foods 41:55–61

Yahfoufi N, Mallet JF, Graham E, Matar C (2018) Role of probiotics and prebiotics in immunomodulation. Curr Opin Food Sci 20:82–91

Recent Trends in Natural Medicines and Nutraceuticals Research

11

Ajaya Kumar Rout, Sujata Dey, Biswanath Patra, Manojit Bhattacharya, Asim Kumar Jana, Bhaskar Behera, and Bijay Kumar Behera

Abstract

Nature is considered as the classic source of natural medicines that are isolated from myriad flora and fauna that exist on earth. Drugs that are derived from natural products are an emerging domain in drug discovery due to their nontoxicity and enhanced health benefits. Nutraceuticals are bioactive components found in natural products consisting of additional nutritional ingredients, which play a key role in the maintenance of health, including the inhibition and sometimes cure of certain diseases. Nutraceuticals are packed with vitamins and essential minerals, purified in various forms such as herbal products, dietary supplements, and food nutrients. Derived the products like phytochemicals, probiotics, antioxidants, vitamins, and essential minerals are generally isolated from microbial and plant sources. The nanotechnological applications have impacted all research areas, including agriculture, science, and health care. Nanoparticles are promising delivery systems toward a specific target. The diverse nanotechnological tools have introduced several nanodelivery systems, including biogenic nanoparticles, nanospheres, and nanocapsules, which improve the availability of bioactive components. The therapeutic prospects of nano-formulated nutraceuticals are enhanced by nanocarriers. Currently, many nanocarrier systems such as liposomes, micelles, nanoemulsions, and polymeric nanoparticles have come into the picture. The amalgamation of several functional properties of nutraceuticals in one particle can contribute toward the multifunctional nanocarrier development, enhancing the efficacy of many curative and

A. K. Rout · S. Dey · B. Patra · A. K. Jana · B. K. Behera (✉)
Aquatic Environmental Biotechnology & Nanotechnology Division, ICAR-Central Inland Fisheries Research Institute, Kolkata, West Bengal, India

M. Bhattacharya · B. Behera
Department of Zoology, Fakir Mohan University, Vyasa Vihar, Balasore, Odisha, India

K. K. Behera et al. (eds.), *Prebiotics, Probiotics and Nutraceuticals*,
https://doi.org/10.1007/978-981-16-8990-1_11

diagnostic protocols. The nanotechnological approaches can resolve the issues regarding low bioavailability, poor permeability and solubility, low absorption in the gastrointestinal tract, and lack of long-term stability of natural medicines.

Keywords

Nutraceuticals · Natural medicines · Nanotechnology · Nanocarriers · Drug delivery

11.1 Introduction

Nowadays, nutraceuticals are frequently utilized as therapeutics. A nutraceutical has the physiological advantage which offers guard against chronic diseases. Nutraceuticals are often used to prevent chronic diseases, slow down the aging process, extend lifespan, promote health, and maintain the construction and operation of body systems. At the moment, nutraceuticals have received substantial interest due to their potential therapeutic, nutritional, and safe properties. According to the recent data, these chemicals have shown promising outcomes against a variety of problems. The goal of this review is to give new perspectives on nutraceuticals based on their disease-modifying applications. The impact of herbal nutraceuticals on hard curative conditions associated with oxidative stress, such as Alzheimer's disease, Parkinson's disease, allergies, diabetes, cardiovascular disease, cancer, eye disease, inflammation, and obesity, has been reported. The newly published papers about diverse characteristics of nutraceuticals as a substitute for pharmaceuticals were found in the literature (Nasri et al. 2014).

Nutraceuticals are the combination of biological substances extracted from natural products by non-denaturing procedures to retain their original potentials without any chemical manipulation. These extracts are assessed in animals and humans to establish their biological properties, as is done with drugs. After their characteristics have been recognized, they are promoted to be used up by humans as part of the diet. Nutraceuticals are naturally available as concentrated extracts which, consumed at a dose higher than that exist in the original foods, have an advantageous effect on health, greater than the natural food itself could possess. These products are authentic due to their good bioavailability and natural origin and can usually be consumed in the long term without any side effects in humans or animals.

Nutraceuticals are causing an enthusiastic deliberation because their concept draws the demarcation between medicine and food. The foremost dissimilarity is based on their derivation, this being artificial for drugs and natural for nutraceuticals. Nutraceuticals occupy the void that exists amid drugs and food and strongly entitle their own permissible space, taking into account their features and particularities and empowering the expansion of their full therapeutic potential (Aronson 2017; Santini et al. 2017; Corzo et al. 2020).

Nutraceuticals have recently gained a lot of attention due to their potential nutritional and medicinal effects. They have shown encouraging results in studies on cardiovascular illnesses (Khosravi-Boroujeni et al. 2012), atherosclerosis (Madihi et al. 2013), diabetes (Baradaran et al. 2013), cancer, (Shirzad et al. 2011), and neurological disorders (Akhlaghi et al. 2011). These situations include many changes, such as alterations in redox state (Baradaran et al. 2014). Most nutraceuticals possess antioxidant properties with the capability to counteract this situation (Parsaei et al. 2013). Henceforth, they are considered as strong foundations for promoting health, especially for prevention of diseases such as gastrointestinal disorders (Kiani et al. 2013), diabetes (Mirhoseini et al. 2013), and renal infection (Bahmani et al. 2013; Rafieian-Kopaie 2013).

A concerted attempt has been made to present new perspectives on nutraceuticals based on their ability to treat disease. The usefulness of herbal nutraceuticals on disorders associated with oxidative stress (such as allergy), obesity, cardiovascular disease, cancer, inflammation, diabetes, Alzheimer's disease, and Parkinson's disease, has been highlighted.

Nanotechnology increases the bioaccessibility and bioavailability of different nutraceuticals for a broad range of clinical applications. Nutraceuticals and bioactive compounds exhibit different hydrophobic and hydrophilic properties. Hydrophilic chemicals are water-soluble and easily absorbed across the lumen of the digestive tract before entering the systemic circulation. Due to their poor solubility nature, long side chains, reactivity, low permeability, and other factors, hydrophobic bioactive compounds have low bioaccessibility and bioavailability. Polyphenols, tocopherols, carotenoids, phytosterols, fatty acids, and fat-soluble vitamins are only a few examples of hydrophobic complexes. This article explores the importance of nanometric delivery systems that assist in increasing the bioavailability and bioaccessibility of hydrophobic bioactive substances. Micelles, reverse micelles, molecular complexes, liposomes, emulsion droplets, solid lipid particles, microemulsions, filled hydrogel particles, biopolymer nanoparticles, and polyelectrolyte complexes are some of the most common delivery methods.

Liposomes are proficient in loading hydrophilic, amphiphilic, and lipophilic compounds and components either in the aqueous core or embedded in the phospholipid bilayer, on the liposome-continuous phase interface (McClements 2019). Microemulsions and micelles are similar in composition, except that microemulsions include both surfactant and carrier lipid, whereas micelles include only surfactant. The micelles get swelled after the addition of carrier lipid. In this situation, swollen micelles and microemulsions are interchangeable.

Nanoemulsions are spherical droplets with a diameter of 100 nanometers that feature a surfactant or protein shell and a lipophilic center. They're good in transporting lipophilic compounds that have been distributed in water (Davidov-Pardo and McClements 2014).

In the upper gastrointestinal system, hydrogels protect oil droplets and control their bioaccessibility, enabling the bioactive compounds to be released in the colon (McClements 2019). In molecular inclusion, a "host" molecule entraps a "guest" bioactive molecule with non-covalent interactions (Joye and McClements 2013). One of the renowned host molecules is 2-hydroxypropyl-β-cyclodextrin (HP-β-CD). The enhanced solubility of the complex, alteration of membrane properties, higher stability of the bioactive molecules, and close proximity of the complex to the intestinal walls act as bioavailability boosters.

11.2 Method

11.2.1 Nanoencapsulation and Nanodelivery of Nutraceuticals

The term "nutraceutical" was first used and created by Dr. Stephen DeFelice in 1989, which is derived from "nutrition" and "pharmaceutical" (Kalra 2003). The World Health Organization defined incorporated probiotics in food products as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Petchsomrit et al. 2017). The use of probiotics inhibits the growth of potentially harmful bacteria and the immunity mechanisms are reinforced. The beneficial properties are improving lactose tolerance, modulating immunity, lowering cholesterol, controlling intestinal infection or prevention and improving iron deficiency (Prasertmanakit et al. 2009). Considering their professed health benefits, probiotics have been combined with a wide range of dairy products, including cheese, yogurt, and frozen dairy desserts. The loss of probiotics during food processing is one of the most significant technological obstacles as the production of stable and viable probiotic cultures is regarded as an immense challenge for the industry (Rodrigues et al. 2012). Apart from the process of food production and storage method, the survival of probiotic microorganisms is strongly influenced by the barriers of human gastrointestinal tract (stomach acid, pancreatic juice, and bile) (Schell and Beermann 2014). Microencapsulation techniques with different matrices are convenient to improve the viability and avert undesirable losses of probiotics and the targeted delivery in the gastrointestinal tract (Anal and Singh 2007).

11.2.2 The Challenge of Incorporating Nutraceuticals into Food

Nutraceuticals are composed of several categories with effective doses of nutraceutical ingredients, to meet the specific health or medical benefits of consumers (Fig. 11.1). But nutraceuticals hold some limitations in their effective incorporation into supplements (Augustin and Sanguansri 2015). The shelf life and properties of the food may be affected by undesirable interactions among nutraceuticals and certain components of the food matrix. Chemically unstable nutraceuticals may be subjected to degradation during storage or digestion. Innumerable delivery mechanisms are designed to transport nutraceuticals to protect them from degradation during processing or by digestive enzymes. This method also improves the stability, covers any kind of undesirable odors and taste, and improves the bioavailability and solubility of nutraceuticals (He and Hwang 2016).

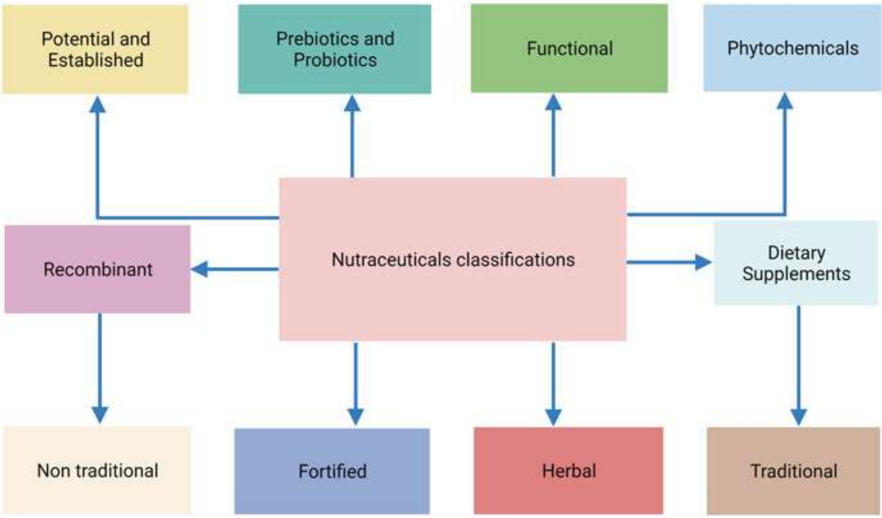


Fig. 11.1 Schematic classification of nutraceuticals

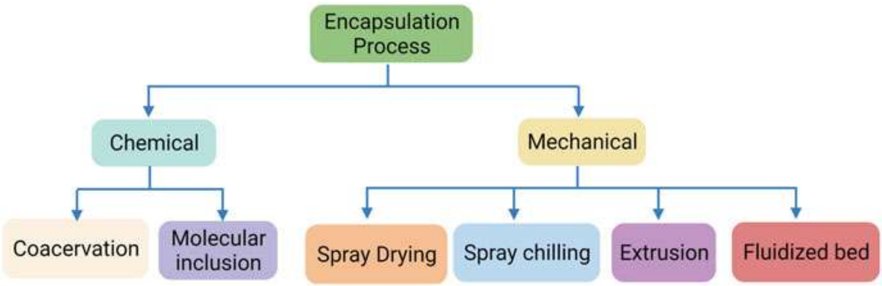


Fig. 11.2 Encapsulation process categories (Carvalho et al. 2016)

11.2.3 Nanoencapsulation and Delivery

The establishment of a delivery system encompasses several steps, from the selection of source materials to the development and commercialization of a final product. Microencapsulation can be divided into two main categories: mechanical processes and chemical processes (Fig. 11.2).

The chemical processes comprise the methods such as cocrystallization, molecular inclusion, coacervation, and interfacial polymerization. The mechanical processes include methods such as spray-chilling/spray-cooling, spray-drying, extrusion, and fluidized bed (Carvalho et al. 2016). The produced microparticles are subjected to further characterization and analysis in terms of physical structure, shape, and controlled release (Estevinho et al. 2016). Especially the controlled release mechanism needs the intervention of a mathematical model to properly design a controlled release system, which ensures the release of a bioactive compound at desired site, at desired time, and at the desired rate. The aforementioned mechanism depends on the nature and quantity of the encapsulating agent, the encapsulation technique, and the environment where the release takes place. The most applicable release mechanisms comprised of dilatation (with gel formation and swelling), osmosis, diffusion, and biodegradation.

11.2.4 Gastroretentive Delivery Systems

Delivery systems falling into this category are created to have a lengthier period of retention in stomach, which improves absorption through the upper gastrointestinal tract. Numerous methods such as mucoadhesion, expansion, floatation, shape modification, and sedimentation have been employed to expand the gastric residence time of nutraceuticals (Murphy et al. 2009). According to Petchsomrit et al. (2017), gastric retention period of curcumin was improved by encapsulation in alginate sponges produced by freeze-drying method. An 8 h retention of encapsulated curcumin in gastric fluid and remaining buoyancy over another 8 h were an amalgamation of alginate:HPMC (4:2) sponges rendered sustained liberation of curcumin up to 71%. Gastroretentive delivery systems were found to be helpful in the treatment of peptic ulcers and gastrointestinal cancer and expanding the availability of inadequately soluble bioactive compounds.

11.2.5 Intestinal Targeted Delivery

Certain compounds are greatly variable and get degraded in gastric fluids. A suitable approach to upsurge their bioavailability and delivery into intestinal conditions can be achieved through using stable biopolymers for efficacious delivery into intestinal system. According to Penalva et al. (2015), folic acid encapsulated in zein nanoparticles is efficiently protected from gastric environment and discharged only at intestinal conditions. Mucoadhesive characteristic of zein's outer surface interacts with mucus layer and epithelium surface for a long time increasing concentration of folic acid in blood for almost 24 hours. The negative charge of protein matrix and folic acid under an alkaline environment permits targeted release in intestine.

11.2.6 Colon-Specific Delivery

Localized treatment of certain bowel diseases such as colon cancer, ulcerative colitis, etc. can be treated with colon-specific systemic transport of proteins and peptides. Delivery systems reliant on pH difference in gastrointestinal tract, pressure difference, and enzyme systems triggered by colonic bacteria are investigated for colon-specific delivery of bioactive substances. For example, colon-specific delivery are achieved by curcumin encapsulated in Eudragit S100, a polymer (pH-sensitive) used as microspheres. A report suggested that only 28.9% of total orally administered curcumin reached the colon after 8 h. More than 80% delivery of curcumin in colon was accomplished when delivered in encapsulated form (Madhavi et al. 2012). This type of colon-specific delivery system can increase the bioavailability of nutraceuticals by overcoming rapid metabolism in the upper gastrointestinal tract.

11.3 Safety Measures, Concerns, and Future Aspects

An important concern of using nanotechnology in the encapsulated delivery system of nutraceuticals is safety. Before a specific delivery system is marketed, proper study for probable risks (allergic and toxic reactions) is mandatory. Nanoparticles act differently in the gastrointestinal tract due to their high surface area and small size (Corona-Hernandez et al. 2013). These properties allow them to pass through physiological barriers (tissue and cell) easily, which may harm biological systems. Additional health risks can be avoided if the tolerable UL (upper intake levels) and RDA (recommended daily allowance) are re-evaluated and re-examined (Cortés-Rojas et al. 2014). Also, a nanoscale delivery system may result in accumulation in human organs or tissues or end up interacting with other compounds. With the continuous advancement of food nanotechnology, verification of the security of all innovative products must be performed. The developers and regulators should be responsible for safety assurance of these novel technologies and products before they are presented to the market. This would be able to raise awareness of public safety concerns regarding the probable influence of nanoscale delivery systems on health. The prodigious potential of nutraceuticals is utilized by establishing encapsulation and delivery of nutraceuticals. The precise mechanism of action and biological effects of some nutraceuticals are still a debatable issue (Watanabe et al. 2013). Further studies are required to develop safe and efficient production. Further future research should be focused on the exploration of the possibility of delivery systems to endorse personalized nutrition and advanced functions of nutraceuticals.

11.4 Conclusion

The biological potential of various components supports the progression of nutraceuticals into new phases aimed at improving patient health. Though this situation is currently under research, it supports the possible management of illnesses such as cancer and diabetes, as well as their negative effects on the skin (photoaging). Algae is one of the most important sources of high-value chemicals, with the potential to prevent oxidation, increase the viability and stability of probiotic bacteria in fermented foods, and improve the texture of meat, fish, and dairy products. However, the other sources remain underexplored. After demonstrating anticancer activity in a single-drug strategy, natural compounds could further enhance the utilization of chemotherapeutic adjuvants or cooperating drugs in combination therapy. Polyphenols are promising molecules for the prevention and treatment of many human pathological conditions (e.g., neurodegenerative diseases).

Avocado, olive oil, fish oil, Docosahexaenoic acid (DHA), phytosterols, psyllium, Eicosapentaenoic acid (EPA), strawberry, flaxseed oil, berberine, nuts (especially pistachio, almonds, and hazelnuts), curcumin, and tea all decreased small dense low-density lipoprotein (sdLDL) levels, low density lipoprotein (LDL) particle size, and LDL particle counts. LDL variations benefit from almost all of the nutraceuticals and particular diet components described above, such as omega-3 fatty acids. The total of silymarin components has the greatest antioxidant capabilities, with concentrations of silymarin flavonoid/flavonolignans, followed by silychristin as one of the strongest antioxidants in the silymarin complex.

Because of the unique properties of nano-sized delivery methods, many nanoparticles have been developed to improve bioavailability for a variety of nutraceuticals and hydrophobic bioactive compounds. As previously stated, understanding the types of interactions that govern colloidal system formation and the conditions in the gastrointestinal tract during digestion can be used to modify a controlled release delivery system that protects and slowly delivers bioactive compounds to a specific absorption site. However, the influence of integrating delivery methods in complex food matrices, as well as the toxicity of such structures, remains unexplored. More study is needed to assess the toxicity and environmental impact of nanoparticle-mediated delivery systems, as well as the effect of the food matrix on the bioavailability of the nanoencapsulated bioactive molecules and the delivery systems' effects on the food matrices. For clinical applications, studies on scaling up the manufacturing process of nanodelivery devices would be critical. Curcumin, coenzyme Q, green tea polyphenols, thymoquinone, quercetin, and other nutraceuticals have been packed as nanoparticles and are effective in "nanochemoprevention" and "nanochemotherapy."

References

Akhlaghi M, Shabanian G, Rafieian-Kopaei M et al (2011) Citrus aurantium blossom and preoperative anxiety. Rev Bras Anestesiol 61:707–712

Anal AK, Singh H (2007) Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. Trends Food Sci Technol 18:240–251

Aronson JK (2017) Defining 'nutraceuticals': neither nutritious nor pharmaceutical. Br J Clin Pharmacol 83:8–19

Augustin MA, Sanguansri L (2015) Challenges and solutions to incorporation of nutraceuticals in foods. Annu Rev Food Sci Technol 6:463–477

Bahmani M, Vakili Saatloo N, Maghsoudi R et al (2013) A comparative study on the effect of ethanol extract of wild Scrophularia deserti and streptomycin on Brucellla melitensis. J HerbMed Pharmacol 2(1):17–20

Baradaran A, Madihi Y, Merrikhi A et al (2013) Serum lipoprotein (a) in diabetic patients with various renal function not yet on dialysis. Pak J Med Sci 29:354–357

Baradaran A, Nasri H, Nematbakhsh M, Rafieian-Kopaei M (2014) Antioxidant activity and preventive effect of aqueous leaf extract of Aloe vera on gentamicin-induced nephrotoxicity in male Wistar rats. Clin Ter 165:7–11

Carvalho IT, Estevinho BN, Santos L (2016) Application of microencapsulated essential oils in cosmetic and personal healthcare products—a review. Int J Cosmet Sci 38:109–119
Corona-Hernandez RI, Álvarez-Parrilla E, Lizardi-Mendoza J et al (2013) Structural stability and viability of microencapsulated probiotic bacteria: a review. Compr Rev Food Sci Food Saf 12: 614–628
Cortés-Rojas DF, Souza CRF, Oliveira WP (2014) Encapsulation of eugenol rich clove extract in solid lipid carriers. J Food Eng 127:34–42
Corzo L, Fernández-Novoa L, Carrera I et al (2020) Nutrition, health, and disease: Role of selected marine and vegetal nutraceuticals. Nutrients 12:747
Davidov-Pardo G, McClements DJ (2014) Resveratrol encapsulation: Designing delivery systems to overcome solubility, stability and bioavailability issues. Trends Food Sci Technol 38:88–103
Estevinho BN, Carlan I, Blaga A, Rocha F (2016) Soluble vitamins (vitamin B12 and vitamin C) microencapsulated with different biopolymers by a spray drying process. Powder Technol 289: 71–78
He X, Hwang H-M (2016) Nanotechnology in food science: Functionality, applicability, and safety assessment. J Food Drug Anal 24:671–681
Joye IJ, McClements DJ (2013) Production of nanoparticles by anti-solvent precipitation for use in food systems. Trends Food Sci Technol 34:109–123
Kalra EK (2003) Nutraceutical-definition and introduction. AAPS PharmSci 5:27–28
Khosravi-Boroujeni H, Mohammadifard N, Sarrafzadegan N et al (2012) Potato consumption and cardiovascular disease risk factors among Iranian population. Int J Food Sci Nutr 63:913–920
Kiani MA, Khodadad A, Mohammadi S et al (2013) Effect of peppermint on pediatrics' pain under endoscopic examination of the large bowel. J HerbMed Pharmacol 2(2):41–44
Madhavi M, Madhavi K, Jithan AV (2012) Preparation and in vitro/in vivo characterization of curcumin microspheres intended to treat colon cancer. J Pharm Bioallied Sci 4:164
Madihi Y, Merrikhi A, Baradaran A et al (2013) Impact of Sumac on postprandial high-fat oxidative stress. Pak J Med Sci 29(1 Suppl):340–345
McClements DJ (2019) Nanoparticle-and microparticle-based delivery systems: encapsulation, protection and release of active compounds. CRC, Boca Raton
Mirhoseini M, Baradaran A, Rafieian-Kopaei M (2013) Medicinal plants, diabetes mellitus and urgent needs. J HerbMed Pharmacol 2(2):53–54
Murphy CS, Pillay V, Choonara YE, du Toit LC (2009) Gastroretentive drug delivery systems: current developments in novel system design and evaluation. Curr Drug Deliv 6:451–460
Nasri H, Baradaran A, Shirzad H, Rafieian-Kopaei M (2014) New concepts in nutraceuticals as alternative for pharmaceuticals. Int J Prev Med 5:1487
Parsaei P, Karimi M, Asadi SY, Rafieian-Kopaei M (2013) Bioactive components and preventive effect of green tea (Camellia sinensis) extract on post-laparotomy intra-abdominal adhesion in rats. Int J Surg 11:811–815
Penalva R, Esparza I, Agüeros M et al (2015) Casein nanoparticles as carriers for the oral delivery of folic acid. Food Hydrocoll 44:399–406
Petchsomrit A, Sermkaew N, Wiwattanapatapee R (2017) Alginate-based composite sponges as gastroretentive carriers for curcumin-loaded self-microemulsifying drug delivery systems. Sci Pharm 85:11
Prasertmanakit S, Praphairaksit N, Chiangthong W, Muangsin N (2009) Ethyl cellulose microcapsules for protecting and controlled release of folic acid. AAPS PharmSciTech 10: 1104–1112
Rafieian-Kopaie M (2013) Metformin and renal injury protection. J Renal Inj Prev 2(3):91–92
Rodrigues D, Sousa S, Gomes AM et al (2012) Storage stability of Lactobacillus paracasei as free cells or encapsulated in alginate-based microcapsules in low pH fruit juices. Food Bioproc Tech 5:2748–2757

Santini A, Tenore GC, Novellino E (2017) Nutraceuticals: a paradigm of proactive medicine. Eur J Pharm Sci 96:53–61

Schell D, Beermann C (2014) Fluidized bed microencapsulation of Lactobacillus reuteri with sweet whey and shellac for improved acid resistance and in-vitro gastro-intestinal survival. Food Res Int 62:308–314

Shirzad H, Burton RC, Smart YC et al (2011) Natural cytotoxicity of NC-2+ cells against the growth and metastasis of WEHI-164 fibrosarcoma. Scand J Immunol 73:85–90

Watanabe F, Yabuta Y, Tanioka Y, Bito T (2013) Biologically active vitamin B12 compounds in foods for preventing deficiency among vegetarians and elderly subjects. J Agric Food Chem 61: 6769–6775

Nutraceuticals: An Alternative of Medicine 12

Rahul Kumar, Sourav Das, Santosh Kumar Sethi,
Pankaj Kumar Parhi, and Birendra Kumar Bindhani

Abstract

In recent years, the increase in demand for enhancing health by nutritive supplements and adequate living standards has been greatly considered in order to decrease medical costs and increase human efficiency and quality of life. Creation of well-being nourishments with bioactive compounds (useful in nourishments) announced as one of the top patterns of the food business as a choice to tranquilize treatment for development of human well-being. The functional food market is large and growing in most of the countries in the world. Functional food production would be cost-effective due to the possibility of launching numerous new functional food products within the existing markets without the large amount of investment. In this emerging market, there is a more interest for scientists and manufacturers to achieve more information about the nutrition and adequate lifestyles. Basically, the impacts and importance of nutraceuticals and functional foods on human health are presented in this chapter. We have summed up the basic related terms to nutraceuticals like functional foods, dietary supplements, designer foods, medical foods, pharmafoods, phytochemicals, and so forth. There are different types of diseases such as

R. Kumar (✉)
Amity Institute of Biotechnology, Ranchi, Amity University Jharkhand, Ranchi, India

S. Das
Indian Institute of Technology, Kanpur, Kanpur, India

S. K. Sethi
Department of Biotechnology, Gangadhar Meher University, Sambalpur, India

P. K. Parhi
Department of Chemistry, Fakir Mohan University, Balasore, India

B. K. Bindhani (✉)
School of Biotechnology, KIIT Deemed to be University, Bhubaneswar, India

K. K. Behera et al. (eds.), *Prebiotics, Probiotics and Nutraceuticals*,
https://doi.org/10.1007/978-981-16-8990-1_12

cardiovascular diseases, obesity, diabetes, cancer, immune system-based diseases, chronic inflammatory disorders, degenerative diseases, various types of depressions, hypertension, memory disorders, etc. which are controlled by functional foods, somehow. The nutrients provided by nutraceuticals are listed in tables. There are many sources of functional foods like fruit and fruit products, vegetables, marine food and their products, spices, etc. that are considered to have health beneficial properties. Additionally, some bioactive compounds and vitamins essential for human body are also discussed. Some compounds like chitin and chitosan are also used as nutraceuticals and functional foods. These compounds have drawn the attentions of researchers in last two decades.

Keywords

Nutraceuticals · Functional foods · Therapeutic effects · Chitin and chitosan · Vitamins · Dietary fibers

12.1 Introduction

Generally, the concept of nutraceuticals was used at about 3000 years ago. In the era of 460–377 BC, Hippocrates (popularly known as the father of modern medicine) quoted: "Let food be thy medicine and medicine be thy food" to forecast a connection among right health food and food products with beneficial effects. Accordingly, the idea of nutraceuticals is not novel, in spite of the fact that it has promoted as of late. The addition of iodine to salt was started in the beginning days of the 1900s by food producers in some countries like the USA. This was the first attempt to prevent goiter disease which represents the nutraceutical concept. In present scenario, scientists have identified more than thousands of compounds in different foods having some medicinal properties. They are exploring new phytochemicals in foods to get some more useful compounds having some disease-preventing properties. In developed countries, they have added nutraceuticals in their daily diet. Now, the consumers are more health conscious, and they explore more information about nutraceuticals and purchasing nutraceuticals-rich food products. Thus, we are availing many food products having many potential health benefits by research and development. So, the Food and Drug Administration is more concerned about these health benefits claimed by different manufacturers across the world (Tamas-krumpe et al. 2020; de Lemos 2020; Thakkar et al. 2020; Harris et al. 2020; Lozano Muñoz and Díaz 2020).

In ancient world, people were using nutraceuticals unknowingly either directly or indirectly. Presently, there is a worldwide interest in "nutraceuticals" because these compounds contribute a significant part in maintenance of health. "Nutraceutical" was used for the first time by uniting the words "nutrition" and "pharmaceutical" in 1989 by Dr. Stephen De Felice. The "nutraceutical" is an edible material which might be a regular food or supplementary food type. Such type of materials is used in medical/health enhancement and in controlling/curing many diseases. Nutraceuticals may be produced by extracted nutritive compounds, dietary supplements, diets, and genetically modified "designer" foods, herbal foods, and packaged foods in the form of cereals, soups, beverages, wine, bear, energy drinks, etc. There are more than a thousand nutraceutical products and processed foods that are commercially available with approved features enhancing health. There are some nutraceutical products which are used in stopping/curing many diseases like cardiac complications and tumor-related issues. There are many similar terms to "nutraceuticals" as follows:

1. Functional foods
2. Dietary supplements
3. Designer foods
4. Medical foods
5. Pharmafoods
6. Phytochemicals

It is noticed that there is a very small difference among all terminologies mentioned above. The term "pharmaceuticals" is used for drugs being used in treating diseases, while the term "nutraceuticals" is used for those products intended to prevent diseases (Emmanuel et al. 2020; Shelke et al. 2020; Díaz et al. 2020a, b; Sato et al. 2020).

1. *Functional Foods*: When the production of food is done exploiting "scientific concepts" with or without the knowledge of how or why it is being used, then such type of food is known as functional food. Thus, functional food is recommended to provide the required number of vitamins, fats, proteins, and carbohydrates which are essential to keep the body healthy. We call the "functional food" as "nutraceutical," when it is given in prevention and/or treatment of disease(s)/disorder(s) other than deficiency conditions like anemia, thus, a functional food for one and might be a nutraceutical for another. There are examples of nutraceuticals such as fortified dairy products (milk acts as nutrient while its product casein is a pharmaceutical product) and citrus fruits (orange juice acts as nutrient while its constituent ascorbic acid is a pharmaceutical product).

2. *Dietary Supplements*: A dietary supplement is a food item which supplements the eating routine that holds at least one constituent like nutrient, natural inorganic elements, a spice, an acidic material, or a basic, biologically active molecules, constituent, distillate, or their mixture.
3. *Designer Foods*: The designer foods are normal food, produced by fortification and nitrification with increased health-promoting properties. These foods are similar in appearance to normal foods and are consumed regularly as a part of diet. There are few examples such as designer egg, designer milk, designer grains, probiotics, prebiotics, designer foods improved with micro- and macronutrients, and designer proteins. Modern biotechnology has adopted bio-fortification in production of designer foods using recombinant DNA technologies and fermentation processes.
4. *Medical Foods*: The clinical nourishments are an uncommon sort of food which are remedial specialists in nature that give dietary administration in a particular infection. For instance, clinical nourishments are the ways to eradicate all the issues of amino acid digestion in affected patients. These days, clinical nourishments are planned to control hyper-homocysteinemia, pancreatic exocrine inadequacy, incendiary issues, malignant growth cachexia, and different illnesses of comparable classes.
5. *Pharma Foods*: A food is produced with a pharmacological additive to improve health, for example, to lower cholesterol level of food. Pharma foods are a new frontier which fills the gap between pharmaceutical and food industries. Basically, pharma foods are different from nutraceuticals such as memory improvement tabs or vitamins as that can’t be bought off by the shelf. A physician/pharmacist needs to distribute these with proper dose and directions to the patients/healthy persons.
6. *Phytochemicals*: Phytochemicals are valuable for human nutrition in the present era. There are any plant phytochemicals recommended to human health like indoles, isothiocyanates, sulforaphane (from broccoli), allyl sulfides (from onions and garlic), and isoflavonoids (from soybeans) (Cong et al. 2020; Pandey et al. 2010).

Thus, recommendations of nutraceuticals are an effort to achieve required therapeutic effects with minimum side effects in comparison with other therapeutic drugs. The inclination for the disclosure and creation of nutraceuticals over pharmaceuticals is very much valued by the drug and pharmaceutical industries. There are few examples of nutraceuticals which are used to treat diseases given below.

1.	Glucosamine	For treatment of arthritis
2.	Lutein	For macular degeneration
3.	Ginseng	For treatment of cold and memory booster
4.	Echinacea	Anti-immune
5.	Folic acid	For treatment of anemia
6.	Cod liver oil capsules	Supplement of vitamins A and D

Some mainstream useful food and refreshment items are eggs and yogurts (rich in omega-3), squeezed orange (calcium), and so forth. Nutraceuticals have been recommended in the following diseases or act as an agent:

1. Useful for heart diseases
2. Obesity reducer
3. Reduce blood glucose
4. Useful for cancer
5. Activate the immune system
6. Useful for inflammation
7. Useful for degenerative diseases
8. Useful for various types of depressions
9. Hypertension
10. Memory booster (Fig. 12.1 and Table 12.1)

12.2 Nutraceuticals and Diseases

Nutraceuticals and functional foods are approved by many regulatory authorities and government as safe having high nutritional value with potential therapeutic effects. These foods contain essential micro- and macronutrients, vitamins, minerals, etc. The list of nutrients and their advantages is given in Table 12.2 (da Silveira Vasconcelos et al. 2020).

We have introduced some diseases in the introduction part. The recommended nutraceuticals for different diseases are listed in Table 12.3.

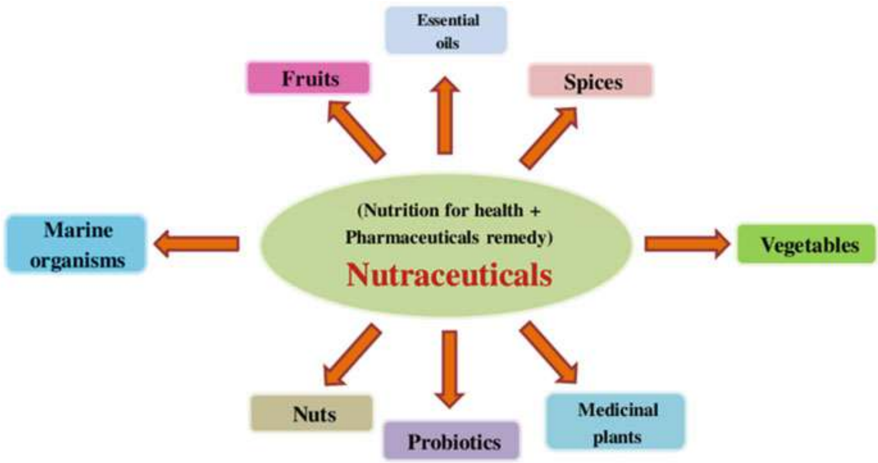


Fig. 12.1 Nutraceuticals and their major wings. (Source: Google Images)

Table 12.1 Type of nutraceuticals, their benefits with respective resources

Sl. no.	Name of bioactive compound	Sources	Health/medical benefits
Carotenoids/isoprenoids			
1.	Alpha-carotene	Carrots and its products	Antioxidants and anticancer
2.	Alpha-cryptoxanthin	Oranges and its products	Keeps the eye healthy
3.	Zea-xanthin	Maize and its products	Reduces chances of cataracts
4.	Beta-carotene	Carrots and leafy vegetables	Keeps the eye protected from UV and ROS scavengers
5.	Lycopene	Tomato, guava, papaya	Antioxidants, anticancer and reduces leukemia
6.	Lutein	Corn, egg, and spinach	Anticancer and keeps the eye healthy in older age
Fibers of diet			
1.	Fibers—soluble	Pulses, barley, and fruits	Anticancer and helps in digestion
2.	Fibers—insoluble	Whole grains (cereals) and nuts	Anticancer and helps in digestion
Phenolic bioactive compounds			
1.	Anthocyanins	All berries	Antioxidants, anti-inflammatory, and antidiabetic
2.	Curcumin	Turmeric	Antioxidants, anticancer, and anti-inflammatory
3.	Flavones	Fruits and vegetables	Antioxidants and anticancer
4.	Flavonones	Fruits (citrus only)	Antioxidants and anticancer
5.	Phenol-derived acids	Pulses and berries	Antioxidants and lowers LDL
6	Resveratrol	Grapes and peanuts	Lowers serum cholesterol
7.	Sterols	Fortified products and stanol-rich foods	Reduces heart attack
8.	Saponins	Soya beans and chickpeas	Anticancer and reduces cholesterol deposition
9.	Ferulic acids	Apples and vegetables	Keeps the eye and heart healthy
10.	Sorbitol	Many fruits	Keeps the bone and teeth healthy
11.	Glucosinolates	Cauliflowers	Anticancer
12.	Thiols	Vegetables	Immune booster
Fatty acid			
1.	PUFA	Flax seeds and salmon	Anti-inflammatory and brain booster
Alkaloids			
1.	Quinone	Cinchona barks	Anti-malaria
2.	Tropane	*Datura*	Keeps the heart healthy
3.	Morphine	Opium	Pain reducer and lowers depressions
4.	Fenugreekine, coumarin, trigonelline, scopoletin	Fenugreek	Helps in hypoglycemia

Table 12.2 List of vitamins and their role in promotion of health (Godhamgaonkar et al. 2020; Atakora et al. 2020; Gupta et al. 2020)

Sl. no.	Name of vitamin	Role in health promotion
1.	Vitamin A	Antioxidant and useful in skin diseases
2.	Vitamin B1	Food digestion and neurological activity
3.	Vitamin B2	Food digestion, eye health, skin disease treatment, catalyzes metabolic reactions
4.	Vitamin B3	Food digestion and brain health
5.	Vitamin B6	Production of genetic contents of cell and RBC, fat, protein, carbohydrate metabolism, and CNS maintenance
6.	Vitamin C	Antioxidant and useful in wound healing
7.	Vitamin D	Promotes strength to bones
8.	Vitamin E	Antioxidant, activation of immune system, production of blood cells
9.	Vitamin K	Helps in blood clotting
Some other essential nutrients		
1.	Calcium	Bone strength, nerve and muscle actions
2.	Iron	Oxygen transportation and synthesis of energy
3.	Folic acids	Effective in heart disease, controls birth defects, RBC production
4.	Phosphorous	Health of bone and teeth, synthesis of DNA
5.	Magnesium	Nerve and muscle function, synthesis of bone, reduces premenstrual syndrome
6.	Iodine	Thyroid maintenance
7.	Copper	Hemoglobin and collagen synthesis, sequester of iron from food, food digestion, and promotion of heart's health
8.	Cobalt	Constituent of vitamin B12 complex, acts as co-enzymes
9.	Chromium	Metabolism of carbohydrate and fats with insulin

12.3 Seafood: A Source of Nutraceuticals

As of now, marine foods are being more popular which in the early days were consumed by coastal region population only. These nourishments pull in shopper by their assortment of flavor, shading, and surface. Numerous shellfishes have well-being-advancing jobs like long-chain omega-3 unsaturated fats. The rundown of nutraceuticals is given in Table 12.4. Lipids, proteins, flavonoids, minerals, carotenoids, catalysts, chitin, and so on are the primary attractant of analysts and nutraceutical enterprises toward shellfishes. The omega-3 unsaturated fats diminish the chances of coronary illness, particular sorts of malignant growth, diabetes, immune system issues, and joint inflammation (Godhamgaonkar et al. 2020) (Fig. 12.2).

Marine Oils: The longer omega-3 fatty acids with high degree of polyunsaturation are commonly known as PUFA, having potential health benefits. These fatty acids are extracted from aquatic resources (algae, fish, marine mammals, etc.), while algae are the main sources of minerals, like iodine, carotenoids,

Table 12.3 List of nutraceuticals and their importance in diseases (Assadpour and Mahdi Jafari 2019; Murti and Mishra 2020; Singh and Rawat 2021)

Sl. no.	Name of disease	Recommendation of nutraceuticals
1.	Heart diseases	Antioxidants
		Fiber-rich food
		Omega-3 poly-unsaturated fatty acids
		Vitamins
		Green vegetables
2.	Obesity	Herbal food product
		Green tea
		Vitamin C-rich food
		Bitter melon
3.	Diabetes	Low fatty acid food
		Lipoic acid-rich food
		Psyllium-rich food lowers glucose content
4.	Cancer	Flavonoid-rich foods
		Phyto-estrogen-rich diet
		Curcumin- and soya-rich foods
5.	Alzheimer's disease	Beta-carotene-, curcumin-, turmeric-, and lycopene-rich food
6.	Parkinson's disease	Vitamin E-rich diet
7.	Inflammatory treatment	Curcumin-, beet root-, cucumber-, and leafy vegetable-rich diet
8.	Pain and arthritis	Fish oil- and curcumin-rich diet

Table 12.4 The bioactive compounds isolated from seafood sources and their importance in the field of nutraceuticals (Ramli et al. 2020; Cheng et al. 2020; Singh and Rawat 2021)

Sl. no.	Compounds	Importance
1.	Chitin	Nutraceutical foods
2.	Chitosan	Biotech industry, agriculture
3.	Glucosamine	Water treatment, juice purification
4.	Carotenoids	Medical food
5.	Caroteno-proteins	Fish food supplement
6.	Omega-3 fatty acids	Baby food formula
7.	Biopeptides	Immune boosters
8.	Minerals	Food supplement
9.	Algae minerals	Food supplement
10.	Chondroitin sulfate	Arthritis and pain killer
11.	Squalene	Skin diseases

xanthophylls, etc. The triacylglycerols are found mainly in body oil and liver oils of fish, which is rich in vitamin A. The long-chain omega-3 unsaturated fats are additionally extricated from lard of marine warm-blooded animals which are rich in eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and

Fig. 12.2 Nutraceuticals from marine food and food products. (Source: Google Images)

docosapentaenoic acid (DPA). The omega-3 unsaturated fats, especially DHA, supervise the unsaturated fat scope of psyche and retina lipids and accept a basic capacity in the improvement of undeveloped organism and children similarly as in the prosperity position and health essentials of pregnant and breastfeeding women (López-Pedrouso et al. 2020; Shaban et al. 2020; Dai et al. 2020).

Chitin and Chitosan: Chitin is obtained from disposes of shrimp, crab, lobster, and crawfish by digestion of protein and breakdown of minerals. On the other hand, the chitosan of various atomic weights is delivered after deacetylation of chitin. The chitosan is soluble in acetic acid and its aqueous mixture. The chitosan ascorbate, chitosan acetic acid derivation, chitosan lactate, and chitosan malate, among others, are solvent in water. Chitosan has numerous medical advantages and energetically utilized in nutraceutical to give well-being-related arrangements (Petriccione et al. 2015) (Fig. 12.3).

12.4 Fruit and Fruit Products: Sources of Nutraceuticals

1. *Annatto fruits*: The annatto (*Bixa orellana* L.) is bearing 10–50 tiny seeds like grape seeds. These are a very good source of natural food color (orange to red). The extracted color is used in coloring cheese, frozen foods, margarine additives, yogurt, meat sauce, butter derived from fish, appetizers, dressings, and sweetening agents. The bixin-rich carotenoids (80% of total) of annatto are a diapocarotenoid and norbixin (a dicarboxylic acid derivative). The carotenoids have many biological activities on broad ranges with health-promoting properties. About 15 minor carotenoids in annatto are reported till now (Noviendri et al. 2011) (Fig. 12.4).

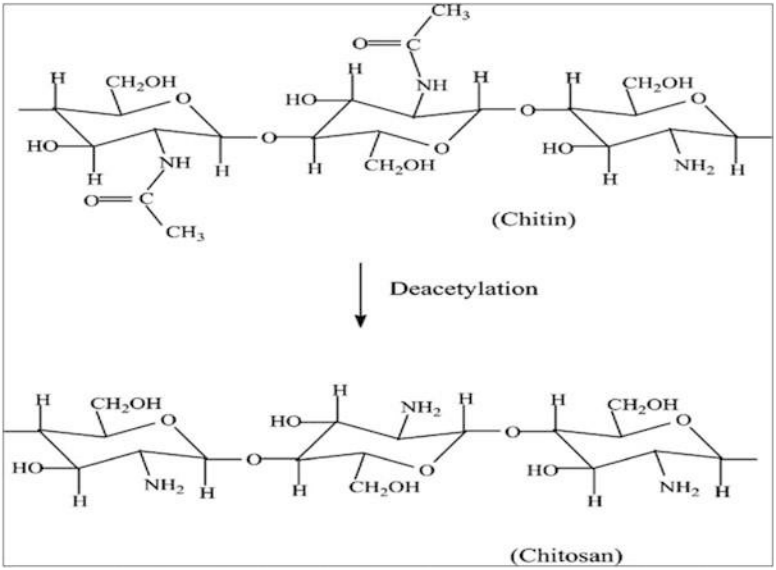


Fig. 12.3 Structure of chitin and chitosan. (Source: Google Images)

2. *Apples*: Apples (*Malus sylvestris*) are used by common population in their daily fruit diet. It is recommended in the treatment of infant diarrhea and every patient. The dried apple powder shows a strong antibacterial activity against *Staphylococcus aureus*, *Streptococcus faecalis*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Researchers reported that apple's phytochemicals (phenolic acids and flavonoids) inhibit the growth of tumor cell in vitro which are natural antioxidants. Apple powder reduces colon carcinogenesis and hepatic metastasis, also (Sut et al. 2019) (Fig. 12.5).
3. *Aronia* fruits: These fruit (*Aronia melanocarpa* Michx.) trees are naturally developed as a strong plant. This belongs to berry family and *Aronia* fruits are berries in black or dark purple color. *Aronia* trees can grow up to 6 feet in height and its fruit ripening takes place during late August or early September. *Aronia* berries are full of anthocyanin which gives colors to the different parts of the plant along with flower and fruits. The dark color is situated all through the berry. Most of the anthocyanin is extracted from its juice by normal crushing. There are mainly four types of anthocyanins found in *Aronia* berries. Among the four types of anthocyanin, 3-substituted monoglycoside is abundant. The constituents of this are given below.

1.	Galactose	68%
2.	Arabinose	28%
3.	Glucose	1.5%
4.	Xylose	2.5%

Fig. 12.4 Annatto fruits. (Source: Google Images)

Fig. 12.5 Apples. (Source: Google Images)

Only galactose and arabinose contribute over 95% of the material. The anthocyanins got from *Aronia* are found in the simplest form out of realm of all plant-derived anthocyanin. *Aronia* organic product-derived drink “chokeberry” having an amazing color and property is used in organic food industry. Its juice has an astringent taste and it has many beneficial properties (like in the case of cranberries). This berry is plentiful in nutrients, minerals, cancer prevention agents, etc. with many advantageous properties. Its juice condensed is appealing to customers. This fruit product refreshment is high in flavonoids, explicitly consolidated tannic acid-rich compounds with anthocyanins, which is proven to have positive benefits in human health and well-being (Shahin et al. 2019) (Fig. 12.6).

4. *Avocados*: Avocado’s (*Persea Americana* Mill.) organic products are utilized as improving specialist like sugar. It is additionally utilized in blend with different

Fig. 12.6 *Aronia* fruits. (Source: Google Images)

Fig. 12.7 Avocado. (Source: Google Images)

organic products, for example, pineapples, oranges, grapefruits, dates, and bananas. This organic product has high substance of lipid around 5–25%. Among the submerged unsaturated fats, the amount of myristic acid may be 0.1%, palmitic 14–21%, and stearic 0.6–1.7%. The oil in the substance is abundant in vitamins A, B, G, and E. The natural item strip is measured as a neutralizing agent poison for vermifuge and an answer for free movements (Tramontin et al. 2020) (Fig. 12.7).

5. *Bananas*: Banana (*Musa acuminata*) is one of the greatest regular item yields of the world. Mainly bananas are part of daily diet nowadays. Its dried form is also consumed in different structures, e.g., banana powder is used as a thickening agent of food and food products. Banana puree is a basic managed thing from the squash of arranged normal things. The puree has a smooth white to mind-blowing yellow tone, liberated from rank. Banana powder is a basic newborn child food. All parts of bananas like strip and tissue have healing utility. Banana squash soup

Fig. 12.8 Bananas. (Source: Google Images)

is taken to control free radicals and the runs and is utilized for treating dangerous ulcers. Antifungal and antibacterial properties are associated with the strip and squash of banana. Norepinephrine, dopamine, and serotonin are in like way extant in the readied bananas. This presents banana as a remedy for rapid heartbeat and frustrating gastric outpouring. The intake of banana enhances the smooth muscle movement of the stomach (Das et al. 2012) (Fig. 12.8).

6. *Bilberries*: This natural product (*Vaccinium myrtillus* L.) is a little dim blue natural product that is eaten fresh as such or made into juice and preserves. Bilberry juice is one of the most anti-mutagenic fruit products and is effective in reducing mutagenicity caused by the polycyclic aromatic hydrocarbons. Gallic acid, an astringent, and an unusual phenolic acid, melilotic acid, are identified in bilberry fruits and leaves. Other phenolic compounds are also found in the plant. Bilberry fruits contain flavonoids (quercitrin, isoquercitrin, hyperoside, avicularin, meratin, and astragaline), catechin, tannins, oligomeric proanthocyanidins, iridoid monoterpenes (asperuloside and monotropein), phenolic acids (chlorogenic, salicylic, and gentisic), quinolizidine alkaloids (myrtine, epimyrtine, and occasionally arbutin), and some pectin. Several anthocyanins are found in the fruits. The pigments are located primarily in the skin of the berries. Bilberry anthocyanins also reduce platelet aggregation in vitro. The results of bilberry are used to treat various conditions coming about in view of diabetes. The raised degrees of glucose in the blood of diabetics trigger various deteriorative events in the body. Bilberry is acknowledged to help improve vision, particularly night vision. Bilberry isolates appear to benefit vision significantly: improving night vision by redesigned recuperation of retinal shades, growing stream inside the vessels of the retina, curbing of Maillard reactions in the point of convergence to diminish cascade advancement, and confirmation from brilliant light. The disease avoidance properties of bilberry concentrates may be liable for these clinical focal points. Cell fortifications have been prescribed to hinder oxidation in the point of convergence and moderate retinal angiopathy that occurs in age-related macular degeneration and diabetic retinopathy. The bilberry has high concentration of tannins which are delicate compounds being used to treat mouth and throat infections (Brasanac-Vukanovic et al. 2018) (Fig. 12.9).

Fig. 12.9 Bilberries. (Source: Google Images)

7. *Black Prunes*: This fruit (*Prunus armeniaca*) is a Japanese apricot. The taste of this fruit is bitter in nature. The key segments of it include glucoside prudomenin, malic acid, and succinic acid. A portion of its therapeutic compounds is helpful in curing and lowering high fever, and destruction of parasitic intestinal worms. Also, which stimulate contraction of the muscles of intestine and gallbladder, results into start secretion of the bile juice. It is additionally an antimicrobial agent. Persons generally use it for the remedy of ongoing runs and loose bowels, hot thirst, achlorhydria, no craving, buildup hacking coughs, persistent fever, biliary ascariasis, hookworms, stomach agony, cholecystitis, and gall stones. The natural products are regularly produced as nibble nourishments or made into a refreshment or wine. There are two particular cultivars: bitter and sweet cultivars. The bitter cultivar is rich in flavor, with more oxalic acid. The sweet cultivar is delicate prepared, genuinely level, with less oxalic acid. The common item pound is considered to pacify biliousness and free radicals and relieve a cerebral pain from superfluous lavishness in alcohol. In India, the prepared natural item is used to end hemorrhages and to ease depleting hemorrhoids. This regular item is recommended as a diuretic in kidney and bladder dissents and is acknowledged to have a helpful effect in the treatment of dermatitis (Shahidi and Naczk 2003) (Fig. 12.10).
8. *Cherimoyas*: Cherimoya (*Annona cherimola*) is generally called custard apple. The substance of prepared cherimoya is most consistently eaten wild. Common items also can be made into juice and plate of blended greens, or blended, or

Fig. 12.10 Black prunes. (Source: Google Images)

Fig. 12.11 Cherimoyas. (Source: Google Images)

desiccated to powder form. The powder of this fruit can be used to control human skin parasites and pneumonia (Mannino et al. 2020) (Fig. 12.11).

9. *Black Dates*: Chinese dates have an extraordinary role in purifying blood stream according to Chinese standard prescription. Some significant therapeutic benefits are to sustain the spleen and stomach, the heart and lungs, and direct various medications. Chinese dates are used for treatment of weak stomach and spleen, paleness, lack of energy (weariness), and salivation. Dates are recommended to be cooked with rice to make a highly nutritious diet (Luna-Vázquez et al. 2013) (Fig. 12.12).

Fig. 12.12 Black dates. (Source: Google Images)

Fig. 12.13 Citrus fruits. (Source: Google Images)

10. *Sour Fruits*: Sour natural products are broadly recognized to cover different sorts of chemo-preventers, for example, limonene, limonoids, glucosides, flavonoids, carotenoids, etc. The citrus juices have hypo-cholesterolemic impacts in coronary illness (Testai and Calderone 2017) (Fig. 12.13).
11. *Oranges*: The orange (*Citrus sinensis*) is a very popular citrus fruit and consumed to maintain the human body's demand of vitamin C. India, Japan, and China are the main producers of oranges in Asia. Traditionally, orange and orange products are recommended to control fever and catarrh. The dried and roasted pulp is crushed to make powder which is used in skin diseases and skin beatification. The fully developed unripen oranges are used to make herbal tea.

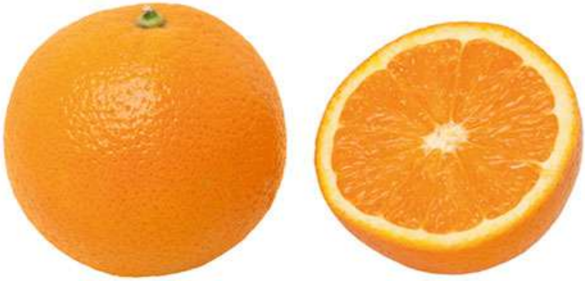


Fig. 12.14 Oranges. (Source: Google Images)

Fig. 12.15 Red tangerines. (Source: Google Images)

This tea is used to relieve acidity, indigestion, and intestinal irregularities (Testai and Calderone 2017) (Fig. 12.14).

12. *Red Tangerine*: Red tangerine (*Citrus reticulata*) fruits are very similar to orange, but the color of pericarp is red toned. This fruit contains high amount of citral, geraniol, linalool, methyl anthranilate, stachydrine, putrescine, pyrocatechol, and glucosides (naringin, poncirin, hesperidin, neohesperidin, and nobiletin). The small quantity of this fruit is recommended as an energy booster, reinforces the lungs, and resolves natural liquid. Red tangerine fruits are utilized to cure chest pain and indigestion and aid in disposing of sputum, etc. Individuals use this fruit in tea making and decoration of different foods with red appearance, in which the decorations combine red tangerine strip and apricot parts. This fruit can also be steamed with chicken and served with alcoholic beverages (Tapas et al. 2008) (Fig. 12.15).

Fig. 12.16 Mandarin oranges. (Source: Google Images)

13. *Mandarin Oranges*: Mandarin oranges (*Citrus reticulate*) can be consumed freshly, mainly used as food additives like gelatin extracts, and used for fruit salads and baking bread and cakes. The oil is produced from dried fruits in off season which is economically very important. Mandarin orange fruits are harsh and bitter to taste after drying. Significant synthetic parts are harsh tasting due to flavone glycosides (neohesperidin, naringin, and neohesperidose), non-bitter flavonoids (hesperidin, rutoside, sinensetin, nobiletin, and tangeretin), various types of oils (like limonene), and gelatin. Some restorative capacities are to address energy dissemination, fortify the spleen, balance unnecessary dampness in the body, and get rid of mucus. Individuals frequently use it to aid in complications in the chest and midsection, disgorging and regurgitating, chest and stomach torments, helpless craving, hacking cough, heartburn, and loose bowels. Likewise, it can also be utilized as a well-known dish called mandarin orange strips hamburger or as saved natural product items to improve the stomach-related framework and blood dissemination (Noviendri et al. 2011) (Fig. 12.16).
14. *Pummelos*: Pummelos (*Citrus maxima*) are the biggest citrus natural product, local to southeastern Asia. The mash and strip have a soothing impact in instances of epilepsy, chorea, and convulsive hacking cough (Pooja, B. 2009) (Fig. 12.17).
15. *Cranberries*: Cranberry (*Vaccinium macrocarpon*) fruits as nutritional complements broadly exist in many types of food and drinks. Its natural products comprise phytochemicals (flavonoids and phenolic acids) which show anticancer properties and also help in various metabolic reactions. The flavonoids found in cranberry are anthocyanins, flavonols, flavan-3-ols, pro-anthocyanidins, etc. Many of these compounds enhance the various metabolic reactions. Richness of anthocyanins is responsible for the attractive red tones of this fruit. Cranberry's natural products additionally contain ellagic acid, which has been appeared to have a wide scope of anti-carcinogenic properties. Cranberries and their product have for a long time been associated with a game

Fig. 12.17 Pummelos. (Source: Google Images)

Fig. 12.18 Cranberries. (Source: Google Images)

plan of clinical inclinations. Cranberries seem to have a generally exceptional menu of sections that have spellbinding power concerning human food, especially in keeping up flourishment and prosperity. Especially cranberry juice and juice-derived products have since quite a while earlier esteemed a notoriety as a treatment for urinary tract complications. Despite the way that the small range pH of the trademark product is supposed as the antimicrobial place, fructose and high atomic weight phenolic mixes have been found to upset the grip of *E. coli* cells in vitro. Fructose and polyphenols forestall mannose-safe associations on certain P-fimbriated *E. coli* separated from joining epithelial tissues in the urinary tract. It is said that cranberries can dress injuries and skin lacrimation, and were utilized on board ships to help forestall scurvy, despite the way that their degree of supplement C is well underneath that of most citrus trademark products. Cranberries are believed to assuage indications of urinary tract contaminations, even forestall their event (Bononi and Tateo 2007) (Fig. 12.18).

16. *Durians*: Durians (*Durio zibethinus* L.) are large fruits protected by rigid, hexangular, short spines. Durian is generally eaten fresh but on the other hand is canned in syrup, or dried, or made into glue. Durian is a decent source of iron,

Fig. 12.19 Durians. (Source: Google Images)

Fig. 12.20 Emblics. (Source: Google Images)

vitamin B, and ascorbic acid. The thick, pudding-like surface of the aril is because of gums, gelatin, and hemicellulose. The substance of durian is said to fill in as a vermifuge. In India, durian products are consumed to fulfill the nutrition requirement of the body, and this fruit keeps our body healthy and energetic (Ho and Bhat 2015) (Fig. 12.19).

17. *Emblics*: Emblics (*Phyllanthus emblica*), both ready and half-ripe fruit products, are used in making jam, jelly, pickles, powder, dry flakes, and squeezed products. This fruit is served along with daily diet in the form of different regular things like chutney. Most commonly, this fruit, known as amla, is used as medication around Asian culture. This is also rich in vitamin C and recommended in stomach remedies. The emblic fruits are regularly observed as diuretic drug and purgative. The emblics when pound and pressed, particularly in the wake of developing, are important for heartburn, sickliness, jaundice, dyspepsia, hacking cough, nasal blockage, backing of pee, and some heart issues. The embolic powder is a sensible expectorant as it engages the bronchial organs (Shah et al. 2011) (Fig. 12.20).

Fig. 12.21 Figs. (Source: Google Images)

18. *Figs*: Figs (*Ficus carica* L.) are generally consumed as is; however, the natural products are likewise prepared as pies, baked goods, treats, or frozen yogurt. The dehydrated form of this fruit is used as diet and energy source because of the presence of high starch content. This fruit is a particularly good source of dietary fiber, which helps in knee joint and various joints movement in human body. Besides, the potassium salts of characteristic acids in figs help keep up destructive stomach settling agent harmony in the body by executing the surplus acids quantity. Dehydrated figs have a productive result on the fundamental systems in the human body. Figs and fig-derived products are being used for remedial purposes, for instance, in curing sarcoma-related issues. Dehydrated figs have for quite a while been recognized for their diuretic activity. The sap is commonly used in getting rid of moles and treating skin cancers and wounds and used as a diuretic and anthelmintic medicine. Moreover, this fruit is used in treatment of throat and nasopharyngeal airway infections. The powder and stem of this fruit plant are used in tumor's affected area and burned area of a human body (Khezri et al. 2016) (Fig. 12.21).
19. *Grapes*: Grapes (*Vitis vinifera*) are administered mainly in wine and beverage industry. Different items incorporate its kernel oil, pomace (solid remaining), hydrocolloids, and anthocyanins. These fruit and fruit products assume a huge function in forestalling or postponing the onset of illnesses including malignant growth and cardiovascular sicknesses. Phenolic compounds and other well-being-advancing compounds are auxiliary plant metabolites that essentially add to the tang and shading attributes of this fruit, their squeezes, and wines. The phenolic compounds of grapes incorporate phenols and its acid derivative, anthocyanins, flavanols, tannins, etc. The flavonoids (C6-C3-C6), which incorporate the anthocyanins, flavonols, and flavan-3-ols, are incredible cancer prevention agents and are observed in more quantity in grape fruit and grape fruit products. Such compounds show a broad scope of biochemical and pharmacological impacts, including calming and anti-allergic activities. Other flavonoids, which are also found in grape fruit like quercetin, kaempferol, and myricetin, additionally repress cancer-causing agent-initiated tumors. The high concentration of anthocyanins in grapes has proven pharmacological properties which are

Fig. 12.22 Grapes. (Source: Google Images)

utilized for therapeutic uses. The mode of administration is oral or IV (intravenous) or IM (intramuscular) infusion, drug arrangements of anthocyanins diminish slim porousness and delicacy. This calming effect of anthocyanins represents their critical anti-edema actions and their applications in diabetic microangiopathy. In similar, it has been accounted for that anthocyanin has antiulcer action and gives security against UV radiation. Ellagic acid and resveratrol are two significant segments to decrease the danger of malignancy and coronary heart infections. Ellagic acid is an acid hydrolytic result of ellagitannin observed in this fruit extract. Resveratrol (3,4,5-trihydroxystilbene), a typically happening phytoalexin conveyed in view of physical damage, has strained a great deal of thought as a utilitarian part. This is observed in huge sums in grape fruit, and its pith in alcoholic beverage is accepted to be liable for reduced death rate from coronary complications in high alcohol-consuming masses. Resveratrol presented to be a sickness chemo-preventive chemical having demonstrated activity in measures addressing three periods of carcinogenesis. It is moreover exhibited to be a cell fortification, stifling lipopolysaccharide or phorbol ester incited superoxide progressive and hydrogen peroxide creation by macrophages. In muscadine grapes, the peels have the most raised assembly of resveratrol (Threlfall et al. 2005) (Fig. 12.22).

20. *Guavas*: Guava (*Psidium guajava* L.) is consumed as fully ripen fresh fruit in various ways like fruit salads, fruit juice, fresh flesh, or pulp. The items produced using young fruit products are generally used to end gastroenteritis and diarrhea in tropical regions. It contains a few glucosides including avicularin, guaijavarin, and amritoside and its derived compound, quercetin. Fruit products of some varieties show high levels of anti-diarrheal, antibacterial, and anti-inflammatory properties, which are antispasmodic, mainly from the impact of the glucosides and their genie and quercetin. The organic product has a slight anti-hyperglycemic effect. Pulp concentrate likewise applies an anti-mutagenic movement and can check the mutagenicity of the immediate activity of mutagens. The guava's flower and juice-based tonic are very effective in heart diseases. The natural product is utilized to cure diarrhea and intense gastrointestinal irregularities (McCook-Russell et al. 2012) (Fig. 12.23).

Fig. 12.23 Guavas. (Source: Google Images)

21. *Hawthorn fruits*: Hawthorn fruits (*Crataegus pinnatifida* Bge.) are used as breakfast and lite food such as hawthorn cookies and hawthorn cake. Hawthorn fruits have a sweet-sour taste and a fresh flavor. The dish of sweet and sour pork with hawthorn is considered a medicinal food, in which hawthorn and licorice are first cooked. It is also used as a sauce for deep-fried pork. The fruit contains chlorogenic acid, caffeic acid, phlobaphene, L-epicatechol, choline, choline acetate, (β)-sitosterol, sorbitol, vitamin C, crategolic acid, hyperin, tartaric acid, citric acid, and certain hormones. Hawthorn is a rich source of flavan-3-ol (α)-epicatechin and proanthocyanidins related to (α)-epicatechin, e.g., epicatechin-(4β→8)-epicatechin (procyanidin B-2). This fruit has also long been used in Chinese herbal medicine to provide one of the best tonic remedies for the heart and circulatory system and for treating swelling. Hawthorn fruits are said to control blood stasis, relieve pains associated with swelling, promote digestive function, and mitigate other conditions, especially in reducing blood pressure. Some of the pharmacological activities, e.g., the hypotensive effects, have been attributed to the chromones. They act in a normalizing way upon the heart, depending on the need, stimulating or depressing its activity. The major medicinal functions are to help digestion, stimulate blood circulation, stop diarrhea, lower blood cholesterol, smooth the surface of the atherosclerotic area, increase blood flow in the heart, increase the myocardial contractibility, and lower blood pressure. Hawthorn products are usually used for treatment of indigestion, infantile marasmus, menstrual cramps, diarrhea, dysentery, hernia, hypercholesterolemia, angina pectoris, and hypertension. The addition of this fruit in daily diet also reduces the level of triglycerides and cholesterol in the human body. Oral administration of natural product indicated viability in bringing down circulatory strain in hypertensive patients. Notwithstanding these

Fig. 12.24 Hawthorn fruits. (Source: Google Images)

human investigations, it was also shown in numerous medicines that the hawthorn products can diminish heart muscle weakness, purify the heart vessels, aid in constriction sufficiency and syphoning power, widen the coronary artery, and circulate blood elegantly to the cardiac tissues. Concentrates of this fruit and their natural products are currently vended in the entire global business sectors as a well-being diet or cardiovascular stimulant (García-Mateos et al. 2013) (Fig. 12.24).

22. *Indian Jujubes*: Indian jujubes (*Ziziphus mauritiana*) prepared normal items are ordinarily eaten unrefined, or braised. Few packaged foods, pulverized, and dehydrated extracts are in like way accessible in the business zones. The regular fruits are commonly utilized for wounds and sores, for aspiratory weights, and for elevation of body temperature. The dehydrated fruit extracts are a smooth diuretic. Now and again the trademark thing pulps are mixed in with salt and bean stew for acid reflux and biliousness (Shahrajabian et al. 2019a) (Fig. 12.25).
23. *Jackfruits*: Jackfruit's (*Artocarpus heterophyllus* L.) tissue is being used in frozen yogurt, chutney, jelly, and jam. The different products of jackfruits are supported to promote packaged food market. Things more captivating than the new squash are called "vegetable meat." The trademark things comparatively are dehydrated and packed. In China, this fruit squash is considered a high-energy diet which has cooling properties and is effective in lowering the impact of liquor in people (Waghmare et al. 2019) (Fig. 12.26).
24. *Kiwifruits*: Kiwifruits (*Actinidia chinensis*) are rich in vitamin C content and normally consumed fresh or as a meal, in plates of blended greens, pies,

Fig. 12.25 Indian jujubes. (Source: Google Images)

Fig. 12.26 Jackfruits. (Source: Google Images)

pudding, and cake filling. Quinic acid and ascorbic acid are fulfilled by adding this fruit and fruit product in fiery daily diets. This fruit holds the proteolytic stimulus "actinidin" which is basically a zymogen and stable on a broad range of pH. Kiwifruit tissue is abundant in folic acid, potassium, chromium, and vitamin E. In China, commonly, crushed kiwifruit is effective in getting rid of kidney or gall stone (Ugolini et al. 2017) (Fig. 12.27).

Fig. 12.27 Kiwifruits. (Source: Google Images)

Fig. 12.28 Loquats. (Source: Google Images)

25. *Loquats*: Loquats (*Eriobotrya japonica*) are customarily considered as a narcotic and are taken to end retching, quench thirst, or mitigate hacking cough (Pereira-Caro et al. 2020) (Fig. 12.28).
26. *Longans*: Longan (*Euphoria longan*) organic products are primarily utilized in the creation of mixed refreshments. The central substances present in this fruit are vitamin B, glucose, sucrose, and tartaric acid. They can be overseen as a stomachic, febrifuge, and vermifuge and are seen as a neutralizing agent for poison. The dehydrated tissue of this fruit is generally used as liquid medicine and for curing drowsiness and neurasthenic mental issues. Some significant remedial limits are to take care of the spleen, build up the heart, and supplement the psyche. For the most part, they are a good remedy for paleness, hyperactive mental development, and heedlessness (Shahrajabian et al. 2019b) (Fig. 12.29).
27. *Lychee*: This fruit (*Litchi chinensis*) can be eaten fresh or dried. Consumed in modest sums, this fruit is generally known to mitigate hacking cough and effectively relieve gastralgia, tumors, and recovery of damaged organs. Aged

Fig. 12.29 Longans. (Source: Google Images)

Fig. 12.30 Lychee. (Source: Google Images)

lychees are likewise utilized in the Chinese medication (Wu et al. 2013) (Fig. 12.30).

28. *Mangoes*: Mango (*Mangifera indica* L.) flesh is used as appetizers or dessert. The fruit juice in dehydrated form is utilized in newborn child and in different nourishments. Mango items have the restorative properties of a purgative, diuretic, and a stuffing specialist as indicated by customary medication. This fruit juice gives a chilling sway and is consumed in boiling temperature in North India. Moreover, it is confirmed to aid/fix cholera and plague. The dehydrated strip and blooms of this fruit, holding up to 15% tannins, are used as astringents in occurrences of free radicals, steady loose bowels, catarrh of the bladder, and progressing urethritis coming about due to gonorrhea (Masibo and He 2009) (Fig. 12.31).
29. *Mangosteens*: Mangosteen (*Garcinia mangostana* L.) is consumed freshly as dessert. The substance adds up to 31% of the organic products. The organic product tissue holds phytin up to 0.68% on a dry premise. The dried organic product powder is utilized to beat looseness of bowels in customary medication and is additionally applied on dermatitis and skin issues, to assuage constant

Fig. 12.31 Mangoes. (Source: Google Images)

Fig. 12.32 Mangosteens. (Source: Google Images)

runs, cystitis, gonorrhea, and gleet; it is sometimes utilized for astringent cream (Garrity et al. 2004) (Fig. 12.32).

30. *Mulberry*: Mulberry (*Morus alba* L.) is common in subtropical domains. Mulberry is bitter and sour, yet has a splendid taste. Some substance parts, for example, morin, dihydromorin, dihydrokaempferol, 2,4,4′,6-tetrahydroxybenzophenone, maclurin, mulberrin, mulberrochromene, and cyclomulberrochromene, have been restricted from this fruit. Critical healing limits are to invigorate kidneys, maintain good eye sight, and support blood circulation. Individuals practice it for curing of tumult and a resting issue,

Fig. 12.33 Mulberries. (Source: Google Images)

hearing loss and blurred eyesight, reducing whitening of hair and facial hair development, stomach related issues, torment in backbone and knee joint, and robustness of tissues and bone joints. This fruit is prepared with rice, chicken, and different decorations, together with red jujubes, lotus seeds, pine seeds, etc. The squash is especially effective against bronchitis, sinusitis, and asthma. It is said to be good for the lungs and is utilized as an antitussive. This is also commonly made into beverages (Lim and Choi 2019) (Fig. 12.33).

31. *Papayas*: Papaya (*Carica papaya* L.) pulp is normally subjected to be eaten fresh with sauce, or salted, or processed as preserves and jam. This fruit is well off in carotenoids, with cryptoxanthin as the majority of which. This fruit can also be made into juice, puree, and nectar. This fruit juice is isolated and then converted into nectar-like edible substance which is consumed as fruit drinks. Papaya juice has a significant, rich orange tone and holds papain. It is similarly high in vitamins A and C and is seen as a "prosperity food." Papaya juice is commonly offered to centers and prosperity food stores in different countries (Santana et al. 2019) (Fig. 12.34).
32. *Passion Fruits*: Organic products of passion fruit (*Passiflora edulis*) substance have less ascorbic corrosiveness than that in the purple tissue yet are more extravagant in all acids (fundamentally citrus extract) and carotene. Carotenoids in the tissue are 0.6–1.16%. The substance is a respectable source of niacin and riboflavin. This fruit juice can be improved and a short time later debilitated with water and further to natural squeezed items and cold beverages. Energy rich fruit concentrate can be used in the preparation of sauces, gelatin-based food items,

Fig. 12.34 Papayas. (Source: Google Images)

Fig. 12.35 Passion fruits. (Source: Google Images)

candy, frozen yogurt, sherbet, cake filling, meringue or chiffon pie, cold natural item soup, and blended beverages. The hardened fruit extract can be put aside for 1 year. The liquid extract of this fruit can moreover be dried out with a desiccator. As demonstrated by Chinese standard prescription, these fruits (or dried powder) can be suggested for lack of sleep, fits, mental emergency, menopause, fevers, strain, and hypertension. It is affluent in nutrients, particularly calcium and magnesium. This fruit and fruit product are consumed to relieve stomach-associated problems and recommended for gastric issues. There is now a correct recuperation of attention in the medication business in the use of glycosides as opiates (Ariharan et al. 2013) (Fig. 12.35).

33. *Persimmons*: Persimmons (*Diospyros kaki* L.) might be added to plates of mixed greens, mixed with frozen yogurt, yogurt, cakes, treats, pastries, desserts, jam, or jelly. In Japan, the dried form of persimmons is consumed as sweets. Dried persimmons have white "persimmon sugar" apparently, with a fragile surface, and a sweet taste. Colossal measures of persimmons are shielded by drying them in the sun. The dehydrated fruits are smoothed into structure by insistent, sugar jewels by then seem on a shallow level. A decoction of the calyx is generally known to alleviate hiccups, hacking coughs, and troubled breathing in Asian nations (Park et al. 2008) (Fig. 12.36).

Fig. 12.36 Persimmons. (Source: Google Images)

34. *Pineapples*: In recent years, this fruit (*Ananas comosus*) has received wider attention as one of the main business tropical organic products on the planet. Field-ready organic products are best eaten fresh. Pineapple juice, nectar, and distillate are presently financially arranged. This fruit juice as syrup is utilized in sugary treats and refreshments or converted to powder form. This fruit is customarily engaged as a diuretic and to assist work, likewise as a wash in instances of sore throat and as a counteractant for nausea (Baba et al. 2016) (Fig. 12.37).
35. *Pomegranates*: This fruit's (*Punica granatum* L.) sacs are edible in the form of red pearls of pomegranates. An alluring shaded juice (purplish red), huge succulent grains, mellow acid sweet taste, and tannin substance of not over 0.25% are the characteristics wanted in the organic products utilized for juice preparation. Pomegranate is a hotspot for cancer prevention agents viewed as anti-atherogenic. The juice is rich in citrus extract and sodium citrate, which can be utilized for drug purposes. Pomegranate juice has been utilized for treating dyspepsia and is viewed as gainful in maintaining the efficiency of the digestive system. Later in vitro examinations showed a critical portion of cancer prevention agent capacity of pomegranate juice against lipid peroxidation in plasma (by up to 33%), in low-thickness lipoprotein (by up to 43%), and in high-density lipoprotein (by up to 22%). Pomegranate juice repressed low-thickness lipoprotein oxidation yet additionally decreased two other related changes of the lipoprotein, i.e., its maintenance to proteoglycan and its helplessness to conglomeration. The antioxidative impacts of this fruit juice in contradiction of lipid peroxidation in entire plasma and in confined lipoproteins have been likewise appeared in vivo in people. Pomegranate juice utilization by people builds the action of their serum paraoxonase, which is high-thickness lipoprotein-related esterase that goes about as an intense defender in contradiction of lipid peroxidation (Johanningsmeier and Harris 2011) (Fig. 12.38).
36. *Sea Buckthorn Fruits*: This fruit (*Hippophae rhamnoides* L.) is an extraordinary and important plant species. Sea buckthorn natural products are among the

Fig. 12.37 Pineapples (Source: Google Images)

Fig. 12.38 Pomegranates. (Source: Google Images)

berries which appear in yellow or orange color, rich in starches, protein, natural acids, amino acids, and nutrients. The substance of these fruits change with organic product development, natural product size, species, and geographic areas. Therapeutic employments of sea buckthorn are all around reported in

Fig. 12.39 Sea buckthorn fruits. (Source: Google Images)

Asia. The main pharmacological capacities ascribed to the sea buckthorn oil include as follows: mitigating effect, antimicrobial activity, help with discomfort, and the advancement of tissue recovery. This fruit oil is additionally suggested as a therapy for oral mucositis, rectum mucositis, vaginal mucositis, cervical disintegration, radiation harm, burns, duodenal ulcers, gastric ulcers, chilblains, skin ulcers brought about by untidiness, and other skin diseases. Sea buckthorn oil removed from seed is well known in corrective arrangements, for example, facial cream. As per the ongoing report from China, in an examination of 350 patients, excellent ointment prepared from this fruit oil had constructive helpful impacts on melanosis, feeble skin wrinkles, and spots. In excess of ten diverse useful nourishments have been created from sea buckthorn natural products in Asia, for example, fluids, powders, fillings, films, glues, pills, ointments, suppositories, and nebulizers. Different items produced using sea buckthorn include refreshments and jam (Bal et al. 2011) (Fig. 12.39).

37. *Santol Fruits*: Santol (*Sandoricum koetjape* Merr.) natural products are typically eaten fresh, once in a while with flavors, in India. The organic products are likewise made into jam and jelly or canned, subsequent to eliminating the seed and stripping. The saved mash is utilized for therapeutic purposes as an astringent (Keen 2001) (Fig. 12.40).
38. *Soursop Fruits*: Soursop (*Annona muricata* L.) natural products are consumed fresh or as liquid extracts in hot territories. The mash is converted into tartlets, jam, syrup, and nectar. The liquid extract of the ready fruits has a diuretic work and is viewed as a remedy for hematuria and urethritis. It is likewise accepted

Fig. 12.40 Santol fruits. (Source: Google Images)

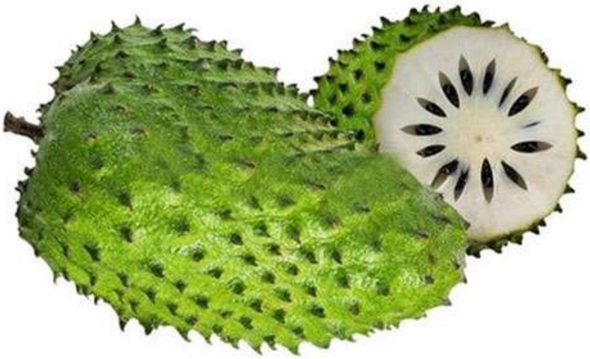


Fig. 12.41 Soursop fruits. (Source: Google Images)

that juice can calm liver afflictions and uncleanliness (Virgen-Cecena et al. 2019) (Fig. 12.41).

39. *Tamarinds*: Tamarind (*Tamarindus indica* L.) natural product's mash is enriched in calcium, phosphorous, iron, thiamine, riboflavin, and niacin. The totally prepared new normal items are relished and eaten fresh. The sensitive, adolescent, and bitter crush is baked and served with rice, fish, and meats in India. The destructive sugary pound is furthermore mixed with sugar to made into dessert, sauce, jam, or nectar. In Asia, an individual use tamarind to save life from highly intense ill. This is also helpful in unconsciousness. This fruit pound is seen as important in the recovery of sense in occurrences of loss of movement (Arshad et al. 2019) (Fig. 12.42).
40. *Wolfberry*: Wolfberry (*Lycium chinense* Miller) is cultivated in subtropical areas. This fruit is pleasant to taste. Wolfberries are red berries which have been used in traditional Asian cuisine in the form of leafy vegetable. This is rich in proteins, vitamins, adequate fiber, low food energy, etc. Wolfberry is recommended to reduce cholesterol and fat and curing serious heart diseases. This is also recommended for diabetic patients as it contains low energy diet. (Chang et al. 2010) (Fig. 12.43).

Fig. 12.42 Tamarind. (Source: Google Images)

Fig. 12.43 Wolfberry. (Source: Google Images)

12.5 Conclusion

Nutraceuticals are food items routed to the public and devoured by ordinary individuals. Accordingly the joint effort and innovation move to different nourishments, and food-related organizations is necessitated. It is assumed that the interest of drug makers and its associated organizations are fundamental for cutting-edge assessments of the impact to and well-being of the human body. Similarly, a commitment course by the International Life Sciences Institute (ILSI) “Useful food genomics” (Functional Food Science and Nutrigenomics) was established by the University of Tokyo graduate school Agricultural Life Science Research Course, for the parts of industrialization and reasonable utilization of nutraceuticals. Around 32 food organizations are taking part in this program, just as industry – the scholarly community ventures driven by Professor Keiko Abe. In particular, each organization is attempting to inspect the varieties and to explain the capacity of the quality articulation level by the admission of food components, for example, proteins and amino acids, which they are keen on creating. They have additionally begun to give general information base connected with the quality articulations grew from food capacities and DNA chips. The business in the scholarly world ventures have increased a ton of considerations worldwide, as it is focused on altogether assessing the general capacity and security of food and food fixings and guaranteeing the establishment for quality plan and quality control. It is basic to acquire information of sickness anticipation, by demonstrating the adequacy in human for creating nutraceuticals. To accomplish, it is important to make groups for the dedicated research in drug advancement, for example, determination of biomarkers and end point of diseases. The exact and target-specific drug is to be developed by scientists and immediately recommended to the affected patient by clinical specialists. Appallingly, as reported by the studies, it is apparent that the country like India falls much behind other developed nations in connection with nutraceutical’s advancement. To grow new practical nourishments which may add to well-being support for individuals and to create actual projects appropriate for different age and ailments, a group framework for mechanical improvement should be made, in particular, to make a data set by utilizing nutrigenomics, for instance, by gathering data about the logical adequacy utilizing biomarkers identified with food. The “General public of Functionality Research of Food and Movement” started obtaining logical information of utilitarian nourishments on human body by gathering different useful nourishments from the business in 2007 and made a decent information base at this point. This is a movement to dissect the capability of nourishments by clinical specialists and scholarly analysts. Presently, there are numerous organizations working in this field around the world. Furthermore, the “Solid Food Material Evaluation Committee” has been framed, in view of the accomplishment of the tasks, for the innovative work, assessment, and production of the data frameworks.

12.6 Future Prospects

In Europe, numerous nations, for example, the Netherlands, France, Germany, Belgium, and Spain, place accentuation on horticulture. The Asian nations are following the way now. India is in serious concern on cutting-edge innovations, as they have a wide scope of information, ability, encounters, and supports, from fundamental examination to industrialization for handling and aging advancements for nourishments, dairy items, and lager. The Nutrigenomics Institute of the sustenance genome research focus in Europe is made for the advancement of nutraceutical enterprises. At the point when nutrigenomics was set up as new sustenance genomic advances for improvement of nutraceuticals, for (1) schooling of post-genomic science, that is, nutrigenomics innovation; (2) advancement and coordination of nutrigenomics advances for commitment to sustenance science in world; (3) application and industrialization of nutrigenomics innovation from the worldwide viewpoints; and (4) foundation of the serious virtual focus of the nutrigenomics innovation. Presently, in excess of 100 associations (college and venture), take an interest in the undertakings as accomplice associations, for the exploration as well as for the advancement of the industrialization. The advancement wherein NuGO is particular is the making of the information base for bioinformatics (nutrient gene data set), concerning the elements of nourishments and its capacity and physiological exercises. This is finished by utilizing genomic innovations and microarray advancements. This information base is planned, from the earliest starting point, to be shared for viable use. The information design is normal for NuGO members. In addition, the examination conventions for information procurement are additionally normalized in a typical configuration, which is successful for the perfect and brief cycle from foundation to industry. The information bases are at present accessible to be shared by the members. In nutraceuticals research in the United States, an emphasis is put on an impeccable methodology, from exploration to industrialization, of nutraceuticals, mostly advanced in Food Valley. In actuality, the United States is zeroing in on the methodologies for avoidance of infections and way of life-related sicknesses dependent on the investigation of nutraceuticals. Some organizations are currently working on a support to give nutraceuticals to individuals who effectively contract sicknesses, through individual hereditary data examination.

Acknowledgments All the authors are thankful to the respective organizations for providing the necessary facility and support to undertake this investigation. The author Rahul Kumar extends sincere acknowledgment to Amity University Jharkhand, Ranchi-834001, Jharkhand, India.

Author Contributions RK, SD, and SKS designed and wrote MS. BKB, PKP, and RK edited the MS.

Competing Interests The authors declare that they have no competing interests.

References

Ariharan VN, Devi VM, Prasad PN (2013) Nutraceutical studies on Passiflora edulis—passion fruit. Int J Paharm Biosci 4(4):176–179

Arshad MS, Imran M, Ahmed A, Sohaib M, Ullah A, Nisa MU, Hina G, Khalid W, Rehana H (2019) Tamarind: A diet-based strategy against lifestyle maladies. Food Sci Nutr 7(11): 3378–3390

Assadpour E, Mahdi Jafari S (2019) A systematic review on nanoencapsulation of food bioactive ingredients and nutraceuticals by various nanocarriers. Crit Rev Food Sci Nutr 59(19): 3129–3151

Atakora LS, Poston L, Hayes L, Flynn AC, White SL (2020) Influence of GDM diagnosis and treatment on weight gain, dietary intake and physical activity in pregnant women with obesity: secondary analysis of the UPBEAT Study. Nutrients 12(2):359

Baba WN, Din S, Punoo HA, Wani TA, Ahmad M, Masoodi FA (2016) Comparison of cheese and paneer whey for production of a functional pineapple beverage: nutraceutical properties and shelf life. J Food Sci Technol 53(6):2558–2568

Bal LM, Meda V, Naik SN, Satya S (2011) Sea buckthorn berries: A potential source of valuable nutrients for nutraceuticals and cosmoceuticals. Food Res Int 44(7):1718–1727

Bononi M, Tateo F (2007) Stabilization of cranberry anthocyanins in nutraceutical capsules. Int J Food Sci Nutr 58(2):142–149

Brasanac-Vukanovic S, Mutic J, Stankovic DM, Arsic I, Blagojevic N, Vukasinovic-Pesic V, Tadic VM (2018) Wild Bilberry (Vaccinium myrtillus L., Ericaceae) from Montenegro as a source of antioxidants for use in the production of nutraceuticals. Molecules 23(8):1864

Chang RCC, Ho YS, Yu MS, So KF (2010) Medicinal and nutraceutical uses of wolfberry in preventing neurodegeneration in Alzheimer's disease. In: Ramassamy C, Bastianetto S (eds) Recent advances on nutrition and the prevention of Alzheimer's disease. Research Signpost, pp 169–185

Cheng SH, Khoo HE, Kong KW, Prasad KN, Galanakis CM (2020) Extraction of carotenoids and applications. In: Carotenoids: properties, processing and applications. Academic, pp 259–288

Cong L, Bremer P, Mirosa M (2020) Functional beverages in selected countries of asia pacific region: a review. Beverages 6(2):21

Dai Y, Lim JX, Yeo SCM, Xiang X, Tan KS, Fu JH, Huang L, Lin H-S (2020) Biotransformation of piceatannol, a dietary resveratrol derivative: promises to human health. Mol Nutr Food Res 64(2):1900905

Das L, Bhaumik E, Raychaudhuri U, Chakraborty R (2012) Role of nutraceuticals in human health. J Food Sci Technol 49(2):173–183

Díaz LD, Fernández-Ruiz V, Cámara M (2020a) An international regulatory review of food health-related claims in functional food products labeling. J Funct Foods 68:103896

Díaz LD, Fernández-Ruiz V, Cámara M (2020b) The frontier between nutrition and pharma: The international regulatory framework of functional foods, food supplements and nutraceuticals. Crit Rev Food Sci Nutr 60(10):1738–1746

Emmanuel BO, Julius OS, Victor KG (2020) Embrace nutraceuticals and live, reject it and embrace death. J Nutr Food Sci 5(1):2

García-Mateos R, Ibarra-Estrada E, Nieto-Angel R (2013) Antioxidant compounds in hawthorn fruits (Crataegus spp.) of Mexico. Rev Mex Biodivers 84(4):1298–1304

Garrity, A. R., Morton, G. A., & Morten, J. C. (2004). Nutraceutical mangosteen composition. 6730333 B1 20040504. US Patent, 7

Godhamgaonkar AA, Wadhwani NS, Joshi SR (2020) Exploring the role of LC-PUFA metabolism in pregnancy complications. Prostaglandins, Leukot Essent Fatty Acids 163:102203

Gupta M, Aggarwal R, Raina N, Khan A (2020) Vitamin-loaded nanocarriers as nutraceuticals in healthcare applications. In: Nanomedicine for bioactives. Springer, Singapore, pp 451–470

Harris CS, Vanderheyden T, Arnason JT (2020) Extraction technologies for plant-derived nutraceuticals and natural health products. In: Spagnuolo PA (ed) Nutraceuticals and human health: the food-to-supplement paradigm, vol 23. Royal Society of Chemistry, London, p 41

Ho LH, Bhat R (2015) Exploring the potential nutraceutical values of durian (Duriozibethinus L.)– An exotic tropical fruit. Food Chem 168:80–89

Johanningsmeier SD, Harris GK (2011) Pomegranate as a functional food and nutraceutical source. Annu Rev Food Sci Technol 2:181–201

Keen CL (2001) Chocolate: food as medicine/medicine as food. J Am Coll Nutr 20(sup5):436S–439S

Khezri S, Dehghan P, Mahmoudi R, Jafarlou M (2016) Fig juice fermented with lactic acid bacteria as a nutraceutical product. Pharm Sci 22(4):260–266

de Lemos M (2020) Use of nutraceuticals in cancer. Evaluation 14:34

Lim SH, Choi CI (2019) Pharmacological properties of Morus nigra L. (black mulberry) as a promising nutraceutical resource. Nutrients 11(2):437

López-Pedrouso M, Lorenzo JM, Cantalapiedra J, Zapata C, Franco JM, Franco D (2020) Aquaculture and by-products: challenges and opportunities in the use of alternative protein sources and bioactive compounds. In: Advances in food and nutrition research, vol 92. Academic, pp 127–185

Lozano Muñoz I, Díaz, NF (2020) Minerals in edible seaweed: health benefits and food safety issues. Crit Rev Food Sci Nutr 1–16

Luna-Vázquez FJ, Ibarra-Alvarado C, Rojas-Molina A, Rojas-Molina JI, Yahia EM, Rivera-Pastrana DM, Rojas-Molina A, Zavala-Sánchez MÁ (2013) Nutraceutical value of black cherry Prunus serotina Ehrh. fruits: antioxidant and antihypertensive properties. Molecules 18(12): 14597–14612

Mannino G, Gentile C, Porcu A, Agliassa C, Caradonna F, Bertea CM (2020) Chemical profile and biological activity of cherimoya (Annona cherimola Mill.) and Atemoya (Annona atemoya) leaves. Molecules 25(11):2612

Masibo M, He Q (2009) Mango bioactive compounds and related nutraceutical properties—a review. Food Rev Intl 25(4):346–370

McCook-Russell KP, Nair MG, Facey PC, Bowen-Forbes CS (2012) Nutritional and nutraceutical comparison of Jamaican Psidium cattleianum (strawberry guava) and Psidium guajava (common guava) fruits. Food Chem 134(2):1069–1073

Murti Y, Mishra P (2020) Synthesis, characterization, and biological evaluation of novel naringenin derivatives as anticancer agents. Curr Bioact Comp 16(4):442–448

Noviendri D, Hasrini RF, Octavianti F (2011) Carotenoids: Sources, medicinal properties and their application in food and nutraceutical industry. J Med Plant Res 5(33):7119–7131

Pandey M, Verma RK, Saraf SA (2010) Nutraceuticals: new era of medicine and health. Asian J Pharm Clin Res 3(1):11–15

Park YS, Leontowicz H, Leontowicz M, Namiesnik J, Jesion I, Gorinstein S (2008) Nutraceutical value of persimmon (Diospyros kaki Thunb.) and its influence on some indices of atherosclerosis in an experiment on rats fed cholesterol-containing diet. Adv Hortic Sci 4:250–254

Pereira-Caro G, Gaillet S, Ordóñez JL, Mena P, Bresciani L, Bindon KA, Rio DD, Rouanet J-M, Moreno-Rojas JM, Crozier A (2020) Bioavailability of red wine and grape seed proanthocyanidins in rats. Food Funct 11(5):3986–4001

Petriccione M, Pasquariello MS, Mastrobuoni F, Zampella L, Di Patre D, Scortichini M (2015) Influence of a chitosan coating on the quality and nutraceutical traits of loquat fruit during postharvest life. Sci Hortic 197:287–296

Pooja, B. (2009) Development of nutraceutical blended beverages from Pummelo (Citrus grandis Linn.). Doctoral dissertation. University of Agricultural Sciences, Bangalore

Ramli NZ, Yahaya MF, Tooyama I, Damanhuri HA (2020) A mechanistic evaluation of antioxidant nutraceuticals on their potential against age-associated neurodegenerative diseases. Antioxidants 9(10):1019

Santana LF, Inada AC, Espirito Santo BLSD, Filiú WF, Pott A, Alves FM, Hiane PA (2019) Nutraceutical potential of Carica papaya in metabolic syndrome. Nutrients 11(7):1608

Sato K, Kodama K, Sengoku S (2020) Corporate characteristics and adoption of good manufacturing practice for dietary supplements in Japan. Int J Environ Res Public Health 17(13):4748

Shaban SN, Mokhtar KI, Ichwan SJA, Al-Ahmad BEM (2020) Potential effects of flaxseed (Linum usitatissimum) in tissue reparative processes: a mini review. J Biomed Clin Sci 5(1):1–7
Shah BN, Seth AK, Modi DC (2011) Fruit and fruit products as functional foods. Int J Food Saf Nutr Publ Health Technol 3(4):14
Shahidi F, Naczk M (2003) Phenolics in food and nutraceuticals. CRC
Shahin L, Phaal SS, Vaidya BN, Brown JE, Joshee N (2019) Aronia (Chokeberry): an underutilized, highly nutraceutical plant. J Med Act Plants 8(4):46–63
Shahrajabian MH, Khoshkharam M, Zandi P, Sun W, Cheng Q (2019a) Jujube, a super-fruit in traditional Chinese medicine, heading for modern pharmacological science. J Med Plants Stud 7(4):173–178
Shahrajabian MH, Sun W, Cheng Q (2019b) Modern pharmacological actions of longan fruits and their usages in traditional herbal remedies. J Med Plants Stud 7(4):179–185
Shelke S, Salunkhe A, Galave V (2020) Health benefits of nutraceuticals: a review. Int J Res Eng Sci Manag 3(5):524–527
da Silveira Vasconcelos M, de Oliveira LMN, Mota EF, de Siqueira Oliveira L, Gomes-Rochette NF, Nunes-Pinheiro DCS, Nabavi SM, de Melo DF (2020) Consumption of rich/enrich phytonutrients food and their relationship with health status of population. In: Nabavi SM, Suntar I, Barreca D, Khan H (eds) Phytonutrients in food. Woodhead, pp 67–101
Singh S, Rawat PS (2021) Nutraceuticals: an approach towards safe and effective medications. In: Hussain A, Behl S (eds) Treating endocrine and metabolic disorders with herbal medicines. IGI Global, pp 278–297
Sut S, Zengin G, Maggi F, Malagoli M, Dall'Acqua, S. (2019) Triterpene acid and phenolics from ancient apples of Friuli Venezia Giulia as nutraceutical ingredients: LC-MS study and in vitro activities. Molecules 24(6):1109
Tamas-krumpe O, Mărgăoan R, Bobiș O, Lațiu C, Urcan A, Ognean L (2020) Natural honey as a potential nutraceutical source. Sci Papers Ser D Anim Sci 1:106–115
Tapas AR, Sakarkar DM, Kakde RB (2008) Flavonoids as nutraceuticals: a review. Trop J Pharm Res 7(3):1089–1099
Testai L, Calderone V (2017) Nutraceutical value of citrus flavanones and their implications in cardiovascular disease. Nutrients 9(5):502
Thakkar S, Anklam E, Xu A, Ulberth F, Li J, Li B, Hugas M, Sarma N, Crerar S, Swift S, Hakamatsuka T, Curtui V, Yan W, Geng X, Slikker W, Tong W (2020) Regulatory landscape of dietary supplements and herbal medicines from a global perspective. Regul Toxicol Pharmacol 16:104647
Threlfall RT, Morris JR, Howard LR, Brownmiller CR, Walker TL (2005) Pressing effects on yield, quality, and nutraceutical content of juice, seeds, and skins from black beauty and sunbelt grapes. J Food Sci 70(3):S167–S171
Tramontin NDS, Luciano TF, Marques SDO, de Souza CT, Muller AP (2020) Ginger and avocado as nutraceuticals for obesity and its comorbidities. Phytother Res 34(6):1282–1290
Ugolini L, Righetti L, Carbone K, Paris R, Malaguti L, Di Francesco A, Micheli L, Paliotta M, Mari M, Lazzeri L (2017) Postharvest application of brassica meal-derived allyl-isothiocyanate to kiwifruit: effect on fruit quality, nutraceutical parameters and physiological response. J Food Sci Technol 54(3):751–760
Virgen-Cecena LJ, Anaya-Esparza LM, Coria-Téllez AV, de Lourdes García-Magaña M, García-Galindo HS, Yahia E, Montalvo-González E (2019) Evaluation of nutritional characteristics and bioactive compounds of soursop-yoghurt and soursop-frozen dessert. Food Sci Biotechnol 28(5):1337–1347
Waghmare R, Memon N, Gat Y, Gandhi S, Kumar V, Panghal A (2019) Jackfruit seed: an accompaniment to functional foods. Braz J Food Technol 22:e2018207
Wu YHS, Chiu CH, Yang DJ, Lin YL, Tseng JK, Chen YC (2013) Inhibitory effects of litchi (Litchi chinensis Sonn.) flower-water extracts on lipase activity and diet-induced obesity. J Funct Foods 5(2):923–929

Check for updates

Socioeconomic Study of Prospective of Probiotics, Prebiotics, and Synbiotics for Sustainable Development of Aquaculture in Indian Sundarbans

13

Swagat Ghosh, Manojit Bhattacharya, and F. H. Rahaman

Abstract

A major marsh and brackish water ecosystem of West Bengal, the Sundarbans, was home to many species of fishes that were the main food source for the people living there. As part of this study, different tools were used in aquaculture extension including a questionnaire survey, participatory rural appraisal, key informant interviews, as well as focus group discussions. The major cultivable species of Sundarbans like mullet, shrimp, tilapia, mud-crabs, giant prawn, and Asian seabass with probiotic, prebiotic, and symbiotic were also assessed. A socioeconomic and technical survey of 40 trained and 40 traditional practitioners of aquaculture were conducted from May 2018 to April 2019. This study and appraisal are intended to the socioeconomic relationship of farmers using probiotic, prebiotic, and synbiotic in aquaculture at Indian Sundarbans. The fish farmers were enriched with much knowledge and information gained from various training, on probiotics. An average benefit-cost ratio of 1:1.89 for traditional fish farmers and 1:3.13 for probiotic users were found. It was helping the modern practitioners to earn more profit to suffice their family needs as well as they were now accomplishing more safety and security measures for their family, investing more on their aquaculture practices. The gross income of the farmers was dependent on basis of the uses of probiotics, conditions of the pond,

S. Ghosh (✉)
Sasya Shyamala Krishi Vigyan Kendra (SSKVK), IRDM Faculty Centre, Ramakrishna Mission Vivekananda Educational and Research Institute (RKMVERI), Kolkata, West Bengal, India

M. Bhattacharya
Department of Zoology, Fakir Mohan University, Vyasa Vihar, Balasore, Odisha, India

F. H. Rahaman
ICAR—Agricultural Technology Application Research Institute (ATARI) Kolkata, Indian Council of Agricultural Research (ICAR), Ministry of Agriculture, Government of India, New Delhi, India

K. K. Behera et al. (eds.), *Prebiotics, Probiotics and Nutraceuticals*,
https://doi.org/10.1007/978-981-16-8990-1_13

engagement of family labor, and fisher family resource endowment. Association with recognized institutions, input support from various government agencies, and other nongovernmental organization might be some point of intervention of the farmers to enhance fish culture in the Indian Sundarbans region.

Keywords

Probiotic · Prebiotic · Aquaculture · Freshwater · Brackish water · Sundarbans · Socioeconomic benefits · India

13.1 Introduction

Probiotics have caused a wide range of beneficial effects on aquatic animals (Dawood et al. 2016; Hoseinifar et al. 2018; Ringo et al. 2018). These additives modulate gastrointestinal microbial communities (Fuller 2012; Hoseinifar et al. 2018) and can affect central nervous system function (Carnevali et al. 2017; Ringo et al. 2018). Accordingly, the use of probiotics in fishes as feed additives can improve the gut microbial community (Gatesoupe 1999; Ghanbari et al. 2015; Ringo et al. 2018), gut morphology (Li et al. 2019), lactic acid production (Ringo et al. 2018), immune system responses (Lazado and Caipang 2014; Li et al. 2019; Nayak 2010), stress tolerance (Carnevali et al. 2017; Mohapatra et al. 2013; Rollo et al. 2006), and production of digestive enzymes (Fuller 2012) and also provide necessary growth factors, vitamins, or amino acids (Lara-Flores 2011; Qin et al. 2014). The relation of these factors can influence reproductive performance in various processes, such as fertility, fertilization, hatching, and development of larvae. Consequently, probiotics can affect the organism's energy metabolism and energy balance, leading to a higher growth rate (Kuebutornye et al. 2019; Qin et al. 2014; Zorriehzahra et al. 2016) and the improvement of reproductive performance (Carnevali et al. 2017). Hence, the proper level of probiotics can lead to the appropriate function of the HPG axis. It has been well documented that these additives can affect the growth and hematological and plasma biochemical parameters of fish. Hematological and plasma biochemical parameters can provide appropriate information on the health status and living conditions of fish (Vani et al. 2011). It has been suggested that dietary supplementation with probiotics can affect biochemical and hematological parameters (Mehrim et al. 2015). Therefore, these variables can provide valuable information regarding the effect of probiotics as an indicator of nutritional status to assess fish health.

Considering the positive nutritional effects of probiotics on the different fish species, the present study aimed to determine the effect of dietary probiotic on the reproductive performance of female rainbow trout broodstock. In this regard, the specific objectives of this study were to determine the effects of dietary probiotic administration on growth performance, hematological and biochemical parameters, and reproductive aspects occurring in this species. It is common for probiotic-based fish farming in Indian Sundarbans, despite its economic importance, to lack data on social, economic, and production characteristics.

In developing nations, declining economic returns from the smallholder system, combined with land fragmentation, has caused a serious imbalance in rural socio-economic stability. Possible alternative livelihoods, in the absence of alternative employment in rural areas, have become a sine qua non for sustaining smallholder livelihoods in many of these agrarian societies. Fishing is practiced domestically by all social classes and can be done by unskilled rural residents in small establishments. Inland ponds offer improved environmental benefits (Ghosh and Sahu 2016) as they offer fisheries a better opportunity to integrate into the landscape. In contrast, if water quality management is to be part of this aquaculture system, individual operators must employ little or no probiotic-based fish farming in comparison with the current practices.

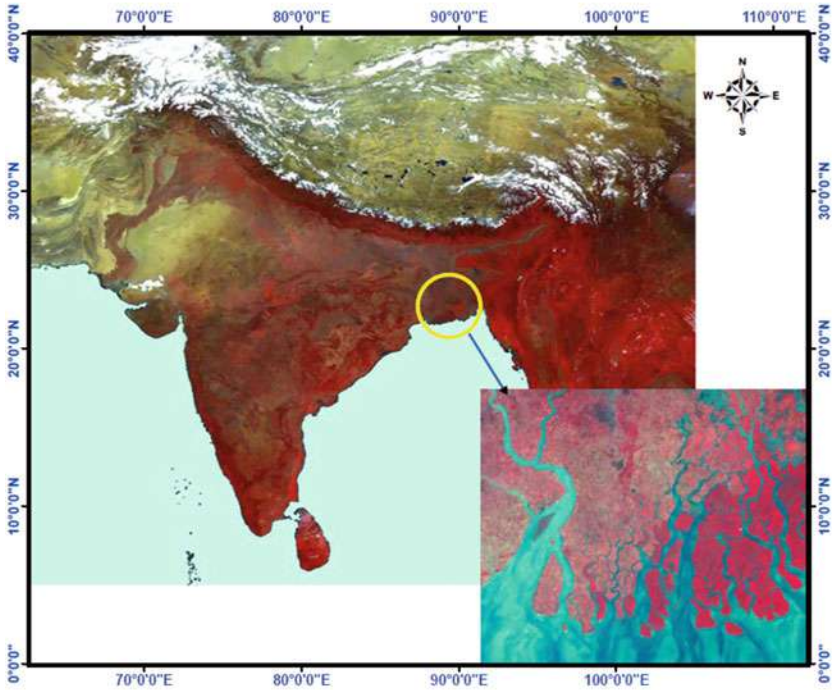
For at least two reasons, it is important to conduct socioeconomic and environmental research on probiotic-based fish farming. Initially, we must define the farmers concerning their socioeconomic status and management approaches so that we can gain an understanding of their practice and identify possible technical deficiencies. In addition, this information will help to determine possible connections between probiotic-based fish farming and the environment, so that technical intervention can be targeted appropriately. A policy framework and extension intervention that recognizes relevant production constraints and productivity factors will be helpful.

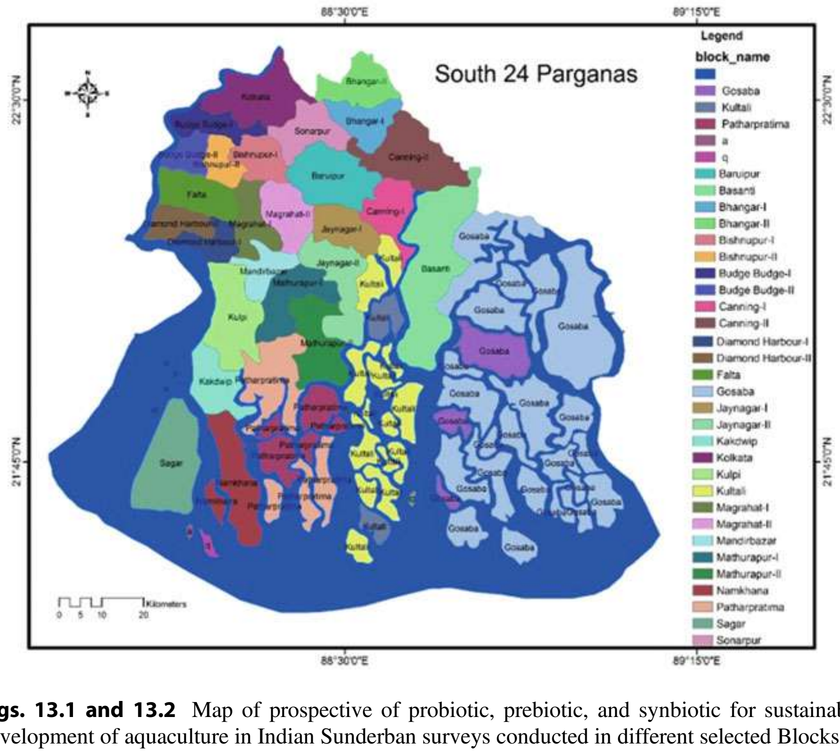
In this study, we evaluated the socioeconomic status of the farmers, the types of production systems, the management strategies adopted, the input level, the level of production, and the net profits of the practicing farmers. Moreover, the paper analyzes the problems of traditional fish farming and the perceived causal links between this culture and environments. On the next page, we describe the factors that have a significant impact on fish culture productivity based on probiotics.

13.2 Materials and Method

13.2.1 Location and Sample Size of the Study

Four blocks in South 24 Parganas District of West Bengal, India, were considered, namely, Gosaba, Basanti, Baruipur, and Kakdwip, which is located in between 21° 29′ 0″N and 22° 33′ 45″N latitude and in between 88° 3′ 45″E and 89° 4′ 50″E longitudes with an average altitude of 7 m from minimum sea level (Figs. 13.1 and 13.2). In South 24 Parganas District, based on the survey data collected in 2011 on probiotic-based fish farming, four major probiotic-based fish farming areas have been identified. A total of three villages were selected from each identified area on the recommendation of the Fisheries Extension Officer and the Agriculture Technology Management Agency. The selection of respondents was based on factors such as the total number of fish farms (20–30), accessibility, demographic factors, livelihood patterns, etc. Survey respondents were drawn from diverse socioeconomic and demographic backgrounds, as well as different genders and occupations connected





Figs. 13.1 and 13.2 Map of prospective of probiotic, prebiotic, and synbiotic for sustainable development of aquaculture in Indian Sunderban surveys conducted in different selected Blocks of South 24 Parganas

with probiotic-based farming. An interview was conducted with 80 respondents representing the cross section of the communities. The inclusion of key informants was based on their familiarity with probiotic-based fish farming and affiliation with extension institutions in the locality.

Using the sample size calculated by Barlett et al. (2001), we conducted the study. It was calculated by the following formula for sample size determination estimating population mean (the yield in t/ha):

$$n = \frac{z^2 \sigma^2}{e^2} \tag{13.1}$$

where n is the sample size, z is the abscissa of the normal curve cutting off an area $\alpha/2$ at the tails (1.96 in our case), "e" is the desired level of precision (±3% ~0.0576 t/ha in our case), and σ is the variance of the variable (yield of fish) in the population (1.0 t/ha in our case). We found a sample size of 66.69–67. For securing a safe sample size, we added 20% sample (13.4) to it. Thus, a sample size of 80 was aimed at.

13.2.2 Variables and Their Measurements

The water area was taken into account when calculating some important techno-economic variables, such as stocking, input use, production, and income. However, ponds, dykes, and water storage ponds were not considered. Several farmers stocked larvae of minute weight, yet some stocked juveniles. This represents gross production. For cases with both fish aquaculture and the whole farm including the dykes, it was calculated the net profits. A cycle period was used to calculate all variables and extrapolate to yearly values. During a cycle, the pond is prepared from the time the first fish seed is planted up to the time the pond is drained. A listing of the variables used in the study and their descriptions is given in Table 13.1.

13.2.3 Method of Data Collection

Using a semi-structured interview schedule that had been pre-tested, farmers were interviewed on their farms. In addition to the interviews, a series of focus group discussions were conducted in conjunction with participatory appraisal techniques. It has been demonstrated that rural livelihoods can be studied with an efficient small-scale survey when it is combined with a variety of tools (Ellis 1998). Therefore, a large variety of techniques and tools such as questionnaire survey, focus group discussion (Holloway 1997), participatory rural appraisal (PRA) (Chambers and Conway 1992), semi-structured interviewing, and documents review (Shiyani and Pandya 1998) was employed in the study.

From May 2018 through April 2019, a semi-structured socioeconomic and technical survey was conducted in different parts of the South 24 Parganas district

Table 13.1 Variables used in the study and their description of probiotic-based fish farming

Sl. no.	Variables	Description
1	Age	Chronological age, measured in years
2	Education	Formal education received; categorized as illiterate, primary, secondary, above secondary
3	Occupation	Primary source of cash income of the household; categorized as agriculture, fishery, business, others (if any)
4	Land ownership	Legal ownership of the pond; categorized as own, leased in, corporate
5	Enterprise ownership	Managerial and ownership nature of the enterprise; categorized as manager and owner and manager
6	Training received	Institutional training on probiotic-based fish farming; if received (yes)—1, otherwise (no)—0
7	Energy source	Source of energy used in the fish farm; categorized as none, electricity, generator
8	Nature of culture	Nature of fish culture; categorized as mutually exclusive “monoculture” and “polyculture”
9	Production system	Categorized as “grow out” and “nursing and grow out”
10	Total pond area	Area of the pond, measured in katha (1 katha = 66.67 m^2)
11	Area of nursery pond	Area of the nursery pond, measured in katha (1 katha = 66.67 m^2)
12	Family size	Number of members in the household
13	Permanent labor	Number of labor staying in the farm during a production cycle for probiotic-based fish farming
14	Casual labor	Number of casual labors employed during a production cycle for probiotic-based fish farming
15	Hired labor	Number of hired labors employed during a production cycle for probiotic-based fish farming
16	Source of water	Water source used for probiotic-based fish farming; categorized as river, lake, reservoir, dam, shallow well, deep well, rainwater, others (if any)
18	Size of probiotic-based fish farming in nursery pond	Size of the fish in the nursery, measured in inch
19	Price of fish in nursing	Last season market price (Rs.) of fish during nursing as recalled by the farmers
20	Weight of fish during transfer	Weight of probiotic-based fish farming during transfer from nursery to main pond; measured in gram
21	Cycle of production	Span of a production cycle for fish production; measured in months
22	Depth of pond	Depth of pond in feet
23	Type of soil	Texture of the soil at the bottom of the pond as reported by the farmers
24	Application of urea	Amount of urea applied in the pond per cycle; measured in kg

(continued)

Table 13.1 (continued)

Sl. no.	Variables	Description
25	Application of potash	Amount of potash applied in the pond per cycle; measured in kg
26	Application of lime	Amount of lime applied in the pond per cycle; measured in kg
27	Application of organic manure	Amount of organic manure applied in the pond per cycle; measured in kg
28	Equipment use	Externally manufactured devices used in probiotic-based fish farming, multiple response recorded against categories: pump, compressor, aerator, vehicles, generators, others (if any)
29	Aerator use	Type of aerator used; categorized as none, paddle wheel at surface, paddle wheel under water
30	Measure water quality	Whether water quality measured in the last cycle; categorized as "yes"—1 and "no"—0
31	Cost of production	Total cost (Rs.) of production of probiotic-based fish farming including input and labor cost per cycle
32	Cost of energy	Cost of energy (Rs.) in probiotic-based fish farming per cycle
33	Harvesting	Technique employed in harvesting fish; categorized as beach seine, cast net
34	Fish production	Physical production of fish per katha per cycle per unit area
35	Income	Cash earning (Rs.) from probiotic-based fish farming in a cycle

of West Bengal. The survey instrument included both closed-ended questions and open-ended ones. These open-ended questions presented information regarding annual production rates, feeding rates, and costs, which are necessary for calculating production rates and net profits for the year. Since Bengali was the vernacular language in the selected study area, all surveys were conducted in Bengali and simultaneously translated into English. In advance of data collection, non-sampled respondents were pre-tested with the questionnaire. From the FGD, data was gathered on aquaculture scenarios, probiotic-based fish farming management, associated economics, and institutional support for probiotic-based fish farming. In inland probiotic-based fish farming, we used the modified PRA tool problem and solution tree, where the trunk of the tree represents the problem, the roots represent its causes, and the branches represent its solutions. Key informant interviews were recorded, and then qualitative statements were transcribed. Complementing the findings from the questionnaire survey were data obtained from secondary data sources kept by fishery departments, focus group discussions, PRAs, and key informant interviews. Key informant interviews were conducted to confirm the validity of the data collected through questionnaires and focus groups.

13.2.4 Data Analysis

Descriptive statistics were analyzed to characterize social, economic, and technical aspects of farming by – frequency distribution (tabular) and estimated average with error bars (graph). Using a linear regression model with 13 independent variables, we identified the factors influencing fish production. The dataset was reduced by using principal component analysis (Jolliffe 2002) to diminish co-linearity among the variables. In a circular rotation (varimax method), a smaller number of highly correlated, yet unidentified variables were combined (Field 2005). Based on Kaiser's criterion, factors exceeding an eigenvalue of one were retained (Kaiser 1970). For analysis, five principal components (PCs) were extracted from the dataset, and the scores were saved. Following linear multiple regression, five PCs were regressed against fish productivity. A descriptive analysis, principal component analysis, and linear regression were carried out using SPSS (version 20.0) software (SPSS Inc., Chicago, USA). Maps were developed using Surfer 8 software.

13.3 Results

Fish farming based on benefits from probiotics was introduced to the coastal blocks of South 24 Parganas district in the early 1990s, mainly using natural seeds without external feeds (Table 13.2). The harvest was used only by the family, and household waste, cow dung, rice, and wheat bran were served as feed. Several diseases were causing outbreaks, and the fish had been growing at different rates. The second half of the twentieth century saw the introduction of traditional and extensive monoculture. Over the past few years, a semi-extensive system of both mono- and polyculture has been practiced, using pellet feeds of mullet, shrimp, tilapia, mud-crab, and giant prawns. Since that time, the local and local export markets have also expanded along with household consumption.

Eighty respondents from the South 24 Parganas district of West Bengal were interviewed: 30 residents of Gosaba, 20 residents of Kakdwip, 16 residents of Basanti, and 14 residents of Baruipur. In sum, the majority of farmers (66.0%) or those who had completed secondary education (30%) had primary education (Fig. 13.2). The primary occupation of the respondents was fishery (50.38%), followed by agriculture (39.7%). Among Kakdwip respondents, fishery-related livelihoods were the most common (57.58%); in Gosaba, agricultural livelihoods were predominant (50.0%). In Bangladesh, the vast majority of fish-rearing farmers (75%) possessed their own land to rear fish, while 22% leased pond space to rear fish. Kakdwip experienced the highest percentage of leases inland (33.33%). The majority (96.3%) of fish growers started the business themselves and managed their farms themselves.

Less than one out of every five farmers (13.75%) received government or nongovernment training on fish rearing (Table 13.3). In Gosaba, more respondents (20%) received training than in the other blocks, and none from Basanti received training. In that study, the majority of farmers (87.5%) practiced Indian major crap

Table 13.2 Timeline on probiotic-based fish farming in the study areas

Time	Culture method	Type of culture	Seeds	Feeds	Marketing	Problems
1990–1995	Traditional	Irregular	Natural	No feeds (pond productivity)	Household use only	Disease outbreak, high differential growth
1996–2000	Traditional and extensive	Monoculture	Natural	Kitchen waste	Local market	Disease outbreak, poor-quality pond water
2001–2005	Extensive	Polyculture	Hatchery and natural	Indigenous low-cost feed	Local market	Poor-quality seeds, disease outbreak
2006–2010	Extensive	Polyculture	Hatchery and natural	Indigenous low-cost feed	Local market and export	Poor seeds, social problem, poaching
2011–2015	Semi-extensive	Monoculture and polyculture both practiced	Hatchery and natural	Indigenous low-cost feed and pellet feed	Local market and export	Giving of poison in the pond, the main problem of probiotic-based fish farming

polyculture, and the rest practiced monoculture. A large proportion of farmers in the study areas practiced grow-out production (96.25%), with only 3.75 % starting just recently. Recent years have seen a rapid increase in the number of homeless individuals in the country. Rain was the most common source of water for farmers (48.67%), followed by river water (18.67%). Fish farmers at Gosaba used rainwater for probiotic-based fish farming, as well as brackish water mixed with fresh water from the river. Rainwater was used by farmers in Baruipur, and deep wells, shallow wells, and dam water were used by farmers in Kakdwip. Basanti, Kakdwip, and Baruipur are the sites with the highest average pond size (between 30 and 40 katha). Several of the ponds were deepest at Gosaba and Baruipur, closely followed by Basanti. A depth range of 6–8 feet was most common. Ponds tended to have silt soils (60%) or clay soils (37.5%). There were clay, silt, and sand-clay bottom soil in Basanti. The soil is mostly clayey or silty in Kakdwip and Baruipur. The most commonly used pieces of farm equipment were pumps (68.95%) and generators (14.29), although aerators (8.05%) and compressors (5.59%) were also found. Water was refilled and discharged from the pond using pump sets. Aerators and generators were also used by farmers in Baruipur and Kakdwip, where resources were relatively more abundant. Both surface paddle wheels (5.06%) and underwater paddle wheels (10.13%) were observed among the aerators. A majority of the farmers (77.5%) are aware of and practice water quality testing. A block farmer in Baruipur monitored water quality on a daily, weekly, biweekly, and monthly basis. Basanti block farmers mostly measure water quality monthly, while very few measure it daily. The majority of farmers in Gosaba and Kakdwip measure water quality weekly and monthly. In selected blocks of South 24 Parganas, the pH of the ponds ranged from 7.1 to 8. Additionally, values between 6–7 and 8 higher were recorded. There was a large percentage of traditional stocking of fish (80%) and a high percentage of beach seine harvesting (75.94%).

In the past, probiotic fish farming employed both permanent employees and casual workers. Permanent labor is mainly employed by large farmers, who receive Rs. 3500–4000 per month. Farm owners employed casual labor according to their needs. Our data showed that Kakdwip had the highest casual labor participation (2–4), followed by Basanti, Baruipur, and Gosaba. However, the highest permanent employment rate (approx. 3 no.) could be observed in Gosaba, followed by Basanti, Baruipur, and Kakdwip.

Few farmers extended the cycle to 10–12 months in order to earn an extra profit. Most farmers completed one cycle within 7–9 months, however. Basanti and Baruipur were the main places where it was observed. Energy was primarily derived from energy purchased from power companies (86.25%) and then from diesel-fired generators (12.5%). In Baruipur, energy costs were at a higher level than in Kakdwip (more than Rs. 400). In Kakdwip, Basanti, and Baruipur blocks, organic manure was applied to the highest degree (15–20 kg). The highest application of urea was in Basanti (3–4 kg), while the highest application of potash was in Kakdwip (3–4 kg). Neither urea nor potash was applied to ponds in Gosaba. It was most expensive in Kakdwip (over Rs. 20,000) followed by Gosaba. In the ponds that were of varying sizes, the net fish production ranged from 70 to 150 kg per cycle. Kakdwip had the

Table 13.3 Distribution of respondents according to selected background variable

	Gosaba	Basanti	Baruipur	Kakdwip	Total	Chi square/exact test significance
Education						
Illiterate	2 (5.71)[a]	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.5)	0.57
Primary	21 (60.0)	9 (64.29)	7 (70.0)	16 (76.19)	53 (66.3)	
Secondary	12 (34.29)	5 (35.71)	2 (20.0)	5 (23.81)	24 (30.0)	
Above secondary	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)	1 (1.2)	
Occupation						
Agriculture	25 (48.08)[b]	14 (50.0)	5 (27.78)	8 (24.24)	52 (39.7)	0.01
Fishery	26 (50.0)	13 (46.43)	8 (44.44)	19 (57.58)	66 (50.38)	
Business	1 (1.92)	1 (3.57)	5 (27.78)	6 (18.18)	13 (9.77)	
Pond ownership						
Owned	33 (73.33)[b]	14 (82.35)	9 (69.23)	21 (66.67)	77 (74.76)	0.84
Leased in	10 (22.22)	3 (17.65)	3 (23.08)	7 (33.33)	23 (22.33)	
Corporate	2 (4.55)	0 (0.0)	1 (7.69)	0 (0.0)	3 (2.91)	
Enterprise ownership						
Manager	2 (5.71)	0 (0.0)	1 (10.0)	0 (0.0)	3 (3.7)	0.36
Owner + Manager	33 (94.29)	14 (100)	9 (90.0)	21 (100)	77 (96.3)	

[a]Figures in parentheses are percentages to the column marginal
[b]Multiple responses recorded

most production, followed by Gosaba. On average, farmers earned between 20,000 and Rs. 50,000 per cycle of growing freshwater fish. By far, the highest incomes were recorded in Kakdwip (over 50,000) and Gosaba. Farmers in Kakdwip received Rs. 30,000 to above 60,000 from the hatchery because it provided quality seeds and 50% of them had a nursing fish pond that could be sold to other blocks in the district.

Since most of the seeds were female, scarcity of male seed was one of the major technical problems of probiotic-based fish farming. Another problem of probiotic-based fish farming was the delay in the start of culture. Since most of the farmers depended on natural seeds and natural seeds were not available all the time, farmers had to delay the commencement of culture. Lastly, and most importantly, social problems like theft and deliberate killing of fish were found to be a menace in the study areas. The questionnaire survey also rated social problems as the most important problem (28.06%) of probiotic-based fish farming, closely followed by low production (26.02%) and disease outbreak (26.02%) (Table 13.4).

Table 13.5 represents the rotated factor (Varimax) matrix of 13 independent variables along with their factor loadings. The communality column shows the variance of individual variables retained in the factors. Variables with high factor loadings and high commonality were considered from the rotated factor matrix. In total, 13 variables were included in PCA, of which 5 principal components with eigenvalues greater than 1 were retained for further analysis. These five PCs explained 67.30% of the total variability in the dataset. Based on the constituent variables, the PC-1 was named as resource endowment. Likewise, other PCs were cycle of production (PC-2), input use (PC-3), family labor (PC-4), and pond condition (PC-5). Table 13.6 represents the multiple regression coefficients of principal components concerning fish productivity in the studied areas. It is observed that resource endowment (PC-1), family labor (PC-4), and pond condition (PC-5) significantly affected the productivity of fish. These PC scores could be termed as good predictors of probiotic-based fish farming in the study areas. The R^2 value of the model was $R^2 = 0.412$ suggesting that five principal components jointly explained 41.2% variance in the fish productivity.

13.4 Discussion

Probiotics are able to improve fish growth parameters, according to recent studies (Ringo et al. 2018). According to Gatesoupe (1999; Ringo et al. 2018), probiotics decrease the pH of the intestine and compete with harmful bacteria to improve immunity and reduce stress in the host. By increasing host health, these factors improve growth parameters. The present study demonstrated that growth parameters improved as probiotic levels increased, and PER and FCR significantly increased and decreased, respectively. These results might be attributed to the positive effects of probiotics, since the best results were observed in the P3 treatment. The increase in digestive activity and stimulation of appetite caused by probiotics can allow the host to grow optimally.

Table 13.4 Distribution of respondents according to selected techno-managerial variables

	Gosaba	Basanti	Baruipur	Kakdwip	Total	Chi square/Fisher's exact sig.
Training received						
Yes	7 (20.0)[a]	0 (0.0)	3 (30.0)	1 (4.76)	11 (13.75)	0.06
No	28 (80.0)	14 (100)	7 (70.0)	20 (95.24)	69 (86.25)	
Nature of culture						
Monoculture	6 (17.14)	1 (7.14)	1 (10)	2 (90.48)	10 (12.5)	0.89
Polyculture	29 (82.86)	13 (92.86)	9 (90)	19 (9.52)	70 (87.5)	
Production system						
Grow out	35 (100)	13 (92.86)	9 (90)	20 (95.24)	77 (96.25)	0.19
Nursing and grow out	0 (0)	1 (7.14)	1 (10)	1 (4.76)	3 (3.75)	
Source of water						
River	20 (37.74)[b]	0 (0)	0 (0)	8 (14.82)	28 (18.67)	0.00
Lake	3 (5.66)	0 (0)	8 (34.78)	2 (3.7)	13 (8.67)	
Reservoir	0 (0)	0 (0)	1 (4.35)	3 (5.56)	4 (2.67)	
Dam	0 (0)	0 (0)	1 (4.35)	6 (11.11)	7 (4.67)	
Shallow well	0 (0)	5 (25.0)	3 (13.04)	7 (12.96)	15 (10.0)	
Deep well	0 (0)	2 (10.0)	0 (0)	8 (14.81)	10 (6.67)	
Rainwater	30 (56.6)	13 (65.0)	10 (43.48)	20 (37.04)	73 (48.67)	
Energy source						
None	0 (0)	0 (0)	1 (10.0)	0 (0)	1 (1.25)	0.04
Electricity	33 (94.29)	13 (92.86)	8 (80.0)	15 (71.43)	69 (86.25)	
Generator	2 (5.71)	1 (7.14)	1 (10.0)	6 (28.57)	10 (12.5)	
Type of soil						
Clay	8 (22.86)	8 (57.14)	5 (50.0)	9 (42.86)	30 (37.5)	0.09
Silt	26 (74.29)	6 (42.86)	4 (40.0)	12 (57.14)	48 (60.0)	
Loam	1 (2.86)	0 (0)	1 (10.0)	0 (0)	1 (2.5)	

(continued)

Table 13.4 (continued)

	Gosaba	Basanti	Baruipur	Kakdwip	Total	Chi square/Fisher's exact sig.
Equipment use						
Pump	58 (74.36)[b]	15 (62.5)	12 (60.0)	26 (68.42)	111 (68.95)	0.23
Compressor	2 (2.7)	2 (8.33)	2 (10.0)	2 (5.26)	9 (5.59)	
Aerator	5 (6.76)	3 (12.5)	2 (10.0)	3 (7.9)	13 (8.05)	
Vehicles	1 (1.35)	2 (8.33)	1 (5.0)	1 (2.63)	5 (3.12)	
Generators	12 (16.23)	2 (8.33)	3 (15.0)	6 (15.79)	23 (14.29)	
Type of aerator used						
None	29 (85.29)	11 (78.57)	9 (81.82)	18 (85.71)	67 (84.81)	0.17
Paddle wheel at surface	4 (11.77)	0 (0)	0 (0)	0 (0)	4 (5.06)	
Paddle wheel under water	1 (2.94)	3 (21.43)	2 (18.18)	3 (14.29)	8 (10.13)	
Measure water quality						
Yes	28 (80.0)	12 (85.71)	7 (70.0)	15 (71.43)	62 (77.5)	0.69
No	7 (20.0)	2 (14.29)	3 (30.0)	6 (28.57)	18 (22.5)	
Stocking of fish						
Traditional	29 (82.86)	13 (92.86)	7 (70)	15 (71.43)	64 (80.0)	0.34
Semi-intensive	6 (17.14)	1 (7.14)	3 (30)	6 (28.57)	16 (20.0)	
Harvesting						
Beach seine	31 (88.57)	12 (85.71)	2 (20)	15 (71.43)	60 (75.94)	0.00
Cast net	4 (11.43)	2 (14.29)	8 (80)	6 (28.57)	19 (24.04)	

[a]Figures in parentheses are percentages to the column marginal
[b]Multiple responses recorded

Table 13.5 Five principal components derived by principal component analysis with loadings for socioeconomic and techno-managerial variables and percent cumulative variance explained

	Rotated component matrix[a]					
	Components					
	1	2	3	4	5	Communality
Age	.036	−−.694	.317	−.240	.100	.652
Total pond area	.691	−.053	−.017	.349	.202	.643
Area of nursery pond	.851	.104	−.134	−.057	−.145	.778
Family size	.197	.150	.066	.709	−.018	.569
Permanent labor in farm	.767	−.163	.091	.174	−.120	.669
Hired labor	.817	.179	−.073	.229	.095	.766
Size of the fish in nursery pond	−.057	−.419	−.207	−.018	.700	.712
Cycle of production	.075	.788	.138	−.147	.137	.686
Depth of pond	.004	.271	.112	.000	.807	.737
Application of inorganic fertilizer	−.070	.189	.774	−.034	.029	.641
Application of lime	−.046	.351	−.568	.328	−.219	.603
Application of organic manure	−.071	−.173	.694	.300	−.222	.655
Cost of production	.233	−.116	−.034	.755	.004	.638
Cumulative variance explained	23.40	37.14	48.55	58.92	67.30	
Eigenvalues	3.04	1.79	1.48	1.35	1.09	

Extraction method: principal component analysis
Rotation method: varimax with Kaiser normalization

Table 13.6 Multiple regression coefficients of principal components in relation to the productivity of probiotic-based fish farming

	Unstandardized coefficients		Standardized coefficients		
	B	Std. error	Beta	*t*	Sig.
(Constant)	89.934	6.813		13.201	.000
PC-1 (resource endowment)	22.714	6.858	.304	3.312	.001
PC-2 (cycle of production)	6.675	6.858	.089	.973	.334
PC-3 (input use)	2.590	6.858	.035	.378	.707
PC-4 (family labor)	39.388	6.858	.526	5.743	.000
PC-5 (pond condition)	−13.698	6.858	−.183	−1.997	.049

R^2 = .412; standard error = 59.39
F-statistic: 9.807; F-sig. = −0.00

Agro-climatic zones in the coastal region of West Bengal have undergone rapid eco-diversification due to climate extremes, unprofitable agriculture, and low employment opportunities (in Mistri 2013; Goswami et al. 2014). Many households of the areas began to farm fish in domestic wastewater ponds that contained probiotics (Ghosh and Sahu 2016). Polyculture has developed from being an input-based system to an extensive commercial system over the last two decades. Small holdings and the lack of market integration opportunities contribute to this trend in many developing countries (Nagothu et al. 2012). Export of agricultural products to external markets coupled with access to non-farm income and institutional credit will likely lead to an intensification of the culture in the near future.

Due to weak extension services in the region, probiotic-based fish farming was not adequately trained in the study areas. In all the study sites where intensive rearing of fish was not feasible, the grow-out system was used to generate fish (Ghosh et al. 2017). Therefore, the system has inherent constraints when it comes to the adoption of certain technologies/inputs and management practices. A diversity of water resources was present in the area, which led to an assortment of varying sources of water. Using probiotics and extension support for fish farming in the region offers location-specific technological solutions (Ghosh et al. 2019). While the use of aerators is on the rise in commercial fish farms, it is still a skewed technology used in probiotic-based fish farming. There was a traditional level of stocking density, while semi-intensive methods were gaining popularity. The study areas had become accustomed to conducting water testing after some training programs. Improvements in management practices are generally not widely adopted. According to literature on Indian context, adoption in the semi-intensive system is high and low in the small subsistence systems (Srinath 2000). A shift in the adoption of scientific culture in this region may be expected given the evolution of probiotic-based fish farming.

As a result of low dissolved oxygen events in early morning, farmers who did not aerate could have had lower production. New (2000) reported that farms using paddle wheel aeration to produce high yields (3.1 t/ha/crop) could be the result of intensive marine farming technology. The number of days between water exchanges and additions was related negatively to production in this survey when using protein-rich diets. Aeration and water exchange were also required to maintain water quality. Water quality can be maintained by continuously exchanging a small proportion of pond water (Ghosh et al. 2017).

For farmers who directly stocked in probiotic fish farming, a lower stocking density may have led to better survival rates. According to Pillay (1990), nursing densities ranged between 50 and 250 nos.m^{-2}, but in our survey, nursing densities ranged from 50 to 250 nos.m^{-2}. As a result of overcrowding and high density, there is the possibility of cannibalism and lowered profits (New and Nair 2002). The animals used by those who direct stocked used juveniles that weighed around 2–3 g, which can tolerate high pH and ammonia (New and Nair 2002). In Thailand, nursing ponds were probably more popular in the early stages of production due to their efficiency and the ease of counting and assessing juvenile fish before releasing them into production ponds (Pillay 1990). The use of batch harvesting reduced predators and competitors, which likely helped improve profits (Newand Nair 2002; Ghosh et al. 2017). Batch harvesting and PL stocking enabled farmers to complete 2.5 cycles a year, while directly stocking juveniles allowed them to complete 5 cycles a year. In response, it was compensated for by lower mortality and shorter time to marketable size (Newand Nair 2002; Ghosh et al. 2017). If the pond is cycled more often, and aeration and feeding are conservative, as well as water treatment and preparation costs, greater net profits are realized.

A principal component analysis of 13 causal variables was performed to simplify the explanation of productivity. This was followed by a regression analysis of probiotic-based fish farming with the principal component (PC) scores. Among the three PCs, we found resource endowment, pond conditions, and family labor to be important for explaining probiotic-based fish productivity. A system's input intensity and management practices are causally linked to its productivity, such as pond size, water access, and the ability to invest in probiotic-based fish farming (New et al. 2010). In contrast, pond conditions can influence the soil and water parameters, as well as aquatic flora and fauna, fish physiological activities, and microbes in water (Munir and Lombardi 2010). In conjunction with management practices, pond conditions determine the production environment (Ghosh et al. 2017). In the context of intensive family labor, management practices, inputs, and most importantly close monitoring of the pond during the whole production cycle will be affected (Shang and Fujimura 1977; Ito 2004; Mandal et al. 2016). Based on this survey, net profits were quite high compared with average village household profits. In addition to market value of the produce, the most important variables positively associated with net profits were probiotic-based fish farming and cost of production (Armstrong et al. 1978). A low cost of cultivation and the reliability of the market led to a handsome return, especially when compared to the villagers who followed subsistence farming or pursued low remuneration jobs as agricultural labor. According to this data regarding management practices and the market, higher net profits may be achieved if conservative feeding and aeration are employed, batch harvesting is used, and a steady market is established.

Fish farming based on probiotics faced several challenges, including adequate supplies of seed in required quantities. Due to the fact that hatcheries supplied the majority of the seed in all locations, this problem was of vital importance. Various obstacles prevented existing hatcheries from being able to produce as much as they could. Because of mass mortality of hatchery-raised seeds, probiotic-based fish farming in study areas may not have produced a high yield. However, by observing the local extension services, other factors that contributed to this were the management of brood stock, the culture of live feed, and management of the diseases. For subsistence systems of probiotic-based fish farming, Dubey et al. (2016) cite seeds and feed scarcity as the major constraints, as well as resource and entitlement constraints. As a result of poor training and insufficient experience, farmers also had a difficult time managing fish diseases. Numerous studies have reported a high prevalence of disease in West Bengal's culture ponds (Bhaumik et al. 1991; Mohan and Bhatta 2002). In addition to theft and poisoning of fish, another major challenge in probiotic-based fish farming has been reported in the literature, especially in South Asian countries (Ahmed et al. 2008a, b).

It was found that efforts had been made to ensure sustainability in probiotic-based fish farming as well as environmental welfare in the communities studied in South 24 Parganas. Microorganisms, such as probiotics, were used by shrimp farmers in Baruipur and Kakdwip not only for lower costs, especially when mixed with molasses, but also for sustainability (Ghosh et al. 2019). Some shrimp farmers have developed a closed farming system based on ponds with higher reserve and treatment ponds and lower stocking densities. The Gosaba survey indicates that two respondents fed their animals commercial feed because they had been suggested commercial feed instead of traditional feed by extension staff. Compared to the other domesticated brood stock sources, the Gangetic brood stock source resulted in lower survival and low-quality offspring. Their lack of adaptation to the conditions prevalent in the study areas may be to blame.

13.5 Conclusion

Probiotic-based fish farming in domestic wastewater ponds has began to emerge as an alternative livelihood option for many households of the coastal West Bengal. The culture is slowly departing from the traditional endogenously managed system to a more extensive and commercial venture. However, use of technology and scientific knowledge have not been satisfactory and ask for critical extension support and access to working capital. The agriculture business, despite operating on a semi-traditional model, has demonstrated moderate productivity and earnings that are significantly higher than their counterparts in not-practicing systems. In the coast of West Bengal, investment in human resource development, tying in with formal financial institutions, and subsidized input support from the fishery department could be some points of intervention for probiotic-based fish farming. Probiotic-based fish farming can also be enhanced through community planning and training sessions. Providing farmers with a forum to discuss environmental issues and manage water supplies could be a purpose of fisheries offices. An early step could be to impose a community-mandated settling system and/or treat ponds, and the following step could be implementing probiotic-based recycling systems.

Acknowledgments The authors are thankful to the farmers, village officials, extension workers, bank officers, and agro-traders that took part in the study.

References

Ahmed N, Van Ahammed F, Brakel M (2008a) An economic analysis of freshwater prawn, Macrobrachium rosenbergii, farming in Mymensingh, Bangladesh. J World Aquac Soc 39(1): 37–50

Ahmed N, Allison EH, Muir JF (2008b) Using the sustainable livelihoods framework to identify constraints and opportunities to the development of freshwater prawn farming in southwest Bangladesh. J World Aquac Soc 39(5):598–611

Armstrong DA, Chippendale D, Knight AW, Colt JE (1978) Interaction of ionized and un–ionized ammonia on short-term survival and growth of prawn larvae, *Macrobrachium rosenbergii*. Biol Bull 154:15–31

Barlett JE, Kotrlik JW, Higgins CC (2001) Organizational research: determining appropriate sample size in survey research. Inf Technol Learn Perform J 19(1):43

Bhaumik U, Pandit PK, Chatterjee JH (1991) Impact of epizootic ulcerative syndrome on the fish yield, consumption and trade in West Bengal. J Inland Fish Soc India 23:45–51

Carnevali O, Maradonna F, Gioacchini G (2017) Integrated control of fish metabolism, wellbeing and reproduction: the role of probiotic. Aquaculture 472:144–155. https://doi.org/10.1016/j.aquaculture.2016.03.037

Chambers R, Conway GR (1992) Sustainable rural livelihoods: practical concepts for the 21st century. IDS Discussion Paper 296. Institute of Development Studies, Brighton, p 296

Dawood M, Koshio S, Ishikawa M, Yokoyama S (2016) Effects of dietary inactivated Pediococcus pentosaceus on growth performance, feed utilization and blood characteristics of red sea bream, Pagrus major juvenile. Aquacult Nutr 22:923–932

Dubey SK, Chand BK, Trivedi RK, Mandal B, Rout SK (2016) Evaluation on the prevailing aquaculture practices in the Indian Sundarban delta: an insight analysis. J Food Agric Environ 14(2):133–141

Ellis F (1998) Survey article: household strategies and rural livelihood diversification. J Dev Stud 35(1):1–38

Field AD (2005) Statistics using SPSS For Windows, 2nd edn. Sage, London

Fuller R (2012) Probiotics: the scientific basis. Springer, Dordrecht, p 398

Gatesoupe FJ (1999) The use of probiotics in aquaculture. Aquaculture 180:147–165. https://doi.org/10.1016/S0044-8486(99)00187-8

Ghanbari M, Kneifel W, Domig KJ (2015) A new view of the fish guts microbiome: advances from next-generation sequencing. Aquaculture 448:464–475. https://doi.org/10.1016/j.aquaculture.2015.06.033

Ghosh S, Sahu NC (2016) Alternative sustainable livelihood opportunities for rural youth men and women empowerment through mud crab farming at Indian Sunderban. Int J Fish Aqu Stud 4(6): 16–19

Ghosh S, Manna S, Sahu NC, Dutta A, Goswami R (2017) Social, economic and production characteristics of freshwater prawn, Macrobrachium rosenbergii (De Man, 1879) culture in West Bengal, India. Aquacult Int 25(5):1935–1957. https://doi.org/10.1007/s10499-017-0165-7

Ghosh S, Manna S, Sahu NC, Rahaman FH, Das. KS (2019) Periphyton based climate smart aquaculture for the farmers of Indian rural Sunderban areas. Indian Res J Ext Edu 19(1):60–72

Goswami R, Chatterjee S, Prasad B (2014) Farm types and their economic characterization in complex agro-ecosystems for informed extension intervention: study from coastal West Bengal, India. Agric Food Econ 2(5). https://doi.org/10.1186/s40100-014-0005-2

Holloway I (1997) Basic concepts for qualitative research. Blackwell Science, Oxford

Hoseinifar SH, Sun Y, Wang A, Zhou Z (2018) Probiotics as means of diseases control in aquaculture, A review of current knowledge and future perspectives. Front Microbiol 9:24–29. https://doi.org/10.3389/fmicb.2018.02429

Ito S (2004) Globalization and agrarian change: a case of freshwater prawn farming in Bangladesh. J Int Dev 16(7):1003–1013

Jolliffe IT (2002) Principal component analysis. Springer, New York

Kaiser HFA (1970) Second generation little-jiffy. Psychometrika 35:401–415

Kuebutornye FK, Abarike ED, Lu Y (2019) A review on the application of Bacillus as probiotics in aquaculture. Fish Shellfish Immunol 87:820–828. https://doi.org/10.1016/j.fsi.2019.02.010

Lara-Flores M (2011) The use of probiotic in aquaculture: an overview. Int Res J Microbiol 2:471–478

Lazado CC, Caipang CMA (2014) Mucosal immunity and probiotics in fish. Fish Shellfish Immunol 39:78–89. https://doi.org/10.1016/j.fsi.2014.04.015
Li X, Ringø E, Hoseinifar SH, Lauzon HL, Birkbeck H, Yang D (2019) The adherence and colonization of microorganisms in fish gastrointestinal tract. Rev Aquac 11:603–618. https://doi.org/10.1111/raq.12248
Mandal S, Burman D, Sarangi SK, Maji B, Bandyopadhyay BK (2016) Homestead production systems in coastal salt-affected areas of Sundarbans: status and way forward strategies. In: Dagar JC, Sharma PC, Sharma DK, Singh AK (eds) Innovative saline agriculture. Springer, New Delhi, pp 371–385
Mehrim AI, Khalil FF, Hassan ME (2015) Hydroyeast Aquaculture® as a reproductive enhancer agent for the adult Nile tilapia (Oreochromis niloticus Linnaeus, 1758). Fish Physiol Biochem 41:371–381. https://doi.org/10.1007/s10695-0149989-5
Mistri A (2013) Migration and sustainable livelihoods: a study from Sundarban biosphere reserve. Asia Pac J Soc Sci 5(2):76–102
Mohan CV, Bhatta R (2002) Social and economic impacts of aquatic animal health problems on aquaculture in India. In: Arthur JR, Phillips MJ, Subasinghe RP, Reantaso MB, Macrae IH (eds) Primary aquatic animal health care in rural, small-scale aquaculture development. FAO Fisheries Technical Paper No. 406. FAO, Rome, pp 63–75
Mohapatra S, Chakraborty T, Kumar V, DeBoeck G, Mohanta K (2013) Aquaculture and stress management: a review of probiotic intervention. J Anim Physiol Anim Nutr 97:405–430. https://doi.org/10.1111/j.1439-0396.2012.01301.x
Munir JF, Lombardi JV (2010) Grow-out systems—site selection and pond construction. In: New MB, Tidwell JH, D'Abramo LR, Kutty MN (eds) Freshwater prawns: biology and farming. Wiley, Chichester, pp 127–153
Nagothu US, Muralidhar M, Kumaran M, Muniyandi B, Umesh NR, Prasad KK, De Silva S (2012) Climate change and shrimp farming in Andhra Pradesh, India: socio-economics and vulnerability. Energy Environ Res 2(2):137
Nayak S (2010) Probiotics and immunity: a fish perspective. Fish Shellfish Immunol 29:2–14. https://doi.org/10.1016/j.fsi.2010.02.017
New MB (2000) History and global status of freshwater prawn farming. In: New MB, Valenti WC (eds) Freshwater prawn culture the farming of *Macrobrachium rosenbergii*. Blackwell Science, Oxford, pp 1–10
New MB (2002) Farming freshwater prawns—a manual for the culture of the giant river prawn (*Macrobranchium rosenbergii*). FAO Fisheries Technical Paper 428. FAO, Rome
New MB, D'Abramo LR, Valenti WC, Singholka S (2010) Sustainability of freshwater prawn culture. In: New MB, Tidwell JH, D'Abramo LR, Kutty MN (eds) Freshwater prawns: biology and farming. Wiley, Chichester, pp 524–530
Pillay TVR (1990) Aquaculture: principles and practices. Fishing News Books, Oxford
Qin C, Xu L, Yang Y, He S, Dai Y, Zhao H, Zhou Z (2014) Comparison of fecundity and offspring immunity in zebrafish fed Lactobacillus rhamnosus CICC 6141 and Lactobacillus casei BL23. Reproduction 147:53–64. https://doi.org/10.1530/REP-13-0141
Ringo E, Hoseinifar SH, Ghosh K, Doan HV, Beck BR, Song S (2018) Lactic acid bacteria in finfish—an update. Front Microbiol 9:1–37. https://doi.org/10.3389/fmicb.2018.01818
Rollo A, Sulpizio R, Nardi M, Silvi S, Orpianesi C, Caggiano M, Cresci A, Carnevali O (2006) Live microbial feed supplement in aquaculture for improvement of stress tolerance. Fish Physiol Biochem 32:167–177. https://doi.org/10.1007/s10695-006-0009-2
Shang YC, Fujimura T (1977) The production economics of freshwater prawn (*Macrobrachium rosenbergii*) farming in Hawaii. Aquaculture 11(2):99–110

Shiyani RL, Pandya HR (1998) Diversification of agriculture in Gujrat: a spatio-temporal analysis. Indian J Agric Econ 53(4):627–639

Srinath K (2000) Experiences in aquaculture: some lessons for extension. Aquaculture development in India: problems and prospects. In: Krishnan M, Birthal PS (eds) Proceedings of the Aquaculture development in India: problems and prospects workshop. National Centre for Agricultural Economics and Policy Research, New Delhi, pp 87–97

Vani T, Saharan N, Mukherjee SC, Ranjan R, Kumar R, Brahmchari RK (2011) Deltamethrin induced alterations of hematological and biochemical parameters in fingerlings of *Catla catla* (Ham.) and their amelioration by dietary supplement of vitamin C. Pesticide Biochem Physiol 101:16–20

Zorriehzahra MJ, Delshad ST, Adel M, Tiwari R, Karthik K, Dhama K, Lazado CC (2016) Probiotics as beneficial microbes in aquaculture: an update on their multiple modes of action: a review. Vet Q 36:228–241. https://doi.org/10.1080/01652176.2016.1172132

Drosophila melanogaster as an In Vivo Model for the Investigation of Host-Microbiota Interaction

14

Swetapadma Sahu, Pragalbha Jaysingh, and Monalisa Mishra

Abstract

Once the microbes entered into the gut, they can alter the physiology of the host. If the microbe is harmful, it results in substandard homeostasis or a pathogenic condition to the host although there are many commensals that reside within the gut. The digestive system previously was found to be the most likely target for the study of gut microbiota and its effects in the gut. The gut microbes exhibit an important role in various physiological processes such as reproduction, nervous, and psychological functions. Thus many microbes are used to treat physiological disorders or diseases. The wide role of microbes from development to treatment warrants further investigation using a model system. There exists structural, developmental, and molecular conservation between various pathways of *Drosophila* and vertebrate gut. Furthermore, various gut microbes and its mode of action for various physiological activities including behavior are also conserved between *Drosophila* and vertebrates. Thus, the mode of action of both advantageous and harmful microbes can be studied using the *Drosophila* model. The current article advocates the *Drosophila* gut as a model to study microbes' interaction by considering structural, functional, immunological, and microbial similarity present between the *Drosophila* and vertebrate gut.

Keywords

Microbes · Probiotics · Neurons · Gut · Neuronal disorder · Neuronal behavior

S. Sahu · P. Jaysingh · M. Mishra (✉)
Neural Developmental Biology Lab, Department of Life Science, National Institute of Rourkela, Rourkela, Odisha, India
e-mail: mishramo@nitrkl.ac.in

K. K. Behera et al. (eds.), *Prebiotics, Probiotics and Nutraceuticals*,
https://doi.org/10.1007/978-981-16-8990-1_14

14.1 Introduction

Our environment is teamed up with both commensals and pathogenic microbes. In humans, the normal microflora accounts for 2 kg of body weight. Microbes are commonly found in various parts of the skin, gastrointestinal tract, respiratory tract, and genitourinary tract. The highest number of microbes (which accounts almost ten times the number of host cells) is present in the skin (i.e., about 10^{12} bacteria/2 m^2 of the area) followed by the gut. Indigenous microbiota of the gut helps in digestion and competes with the infectious microbes for their survival. They get energy from the oxidation of undigested carbohydrates for their survival. In turn, they enhance fatty acid absorption for the host and synthesize essential vitamins and toxins as their secondary metabolite which enhances growth and metabolism (Cummings and Macfarlane 1997). Ultimately the microbes contribute to maintaining the gut homeostasis. Thus, any imbalance in the microbial fauna leads to gastrointestinal infection and metabolic imbalances leading to a pathogenic condition. How do microbes enter into the gut? The entry of microbes into the gut begins immediately after birth. Usually, the skin of the mother gets in contact with the child during breast-feeding. So microbes find their ways to enter into the gut (Liévin-Le Moal and Servin 2014). Here the study focuses on the maintenance of various physiological systems and treatment associated with diseases with the help of gut microbes. Literature provides evidence of model organisms to establish the role of gut microbe in the treatment of diseases. This chapter summarizes the use of model organism *Drosophila* to study gut-microbe interaction.

In comparison with that of the mammalian microbiota, *Drosophila* possesses the less diversified type of microorganisms (Wong et al. 2016). Mammals possess 500 different types of microbial species in their body (Broderick and Lemaitre 2012) which is greater than that of the fly. Fly possesses only 30 different species of microbes. These features of *Drosophila* provide us the advantage to be a model in the study of application-based research concerning microorganisms. Adult *Drosophila* contains about 10^5 number of bacteria (Engel and Moran 2013; Ryu et al. 2010). The microbes that are prevalent in *Drosophila* are especially three types of species, namely, *Lactobacillus* (Gram-positive facultative anaerobic rods), *Acetobacter* (Gram-negative aerobic rods), and *Enterococcus* (Gram-positive facultative anaerobic cocci). All of these constitute about 85% of total bacterial community and the other 15% are made up of *Micrococcus*, *Serratia*, *Pseudomonas*, and *Erwinia* (Chandler et al. 2011). In establishing all types of physiological, developmental, as well as well-developed larval features, microbiota plays a vital role. For example, developmental time points and larval sizes are mostly affected by *A. pomorum* (Shin et al. 2011). An infection of *Enterobacter ludwigii* alters the physiology and metabolism (Priyadarsini et al. 2020) and causes neurodegenerative disease in *D. melanogaster* (Priyadarsini et al. 2019).

14.2 A *Drosophila* Approach to Study the Defects in the Gut

Drosophila melanogaster is frequently used to investigate the metabolism of the human body. Reasons which ensure us to use *Drosophila* as a model (Trinder et al. 2017) for the current study are given below:

1. It has structural and functional similarities with the mammalian gut (Lemaitre and Miguel-Aliaga 2013).
2. Various signaling pathways like Toll and Toll-like receptor signaling pathways are conserved between *Drosophila* and vertebrate (Iwasaki and Medzhitov 2010; Takeuchi and Akira 2010).
3. *Drosophila* provides information for the defense against the gut as well as intestinal microbiota.
4. The defense mechanism of *Drosophila* during microbe attack shares similarity with vertebrates.
5. Genes involved with immune responses like signaling, gene expressions, metabolism, connecting the immune systems with other organs and systems, tissue homeostasis, gut physiology, development, and metabolism are conserved between *Drosophila* and vertebrate (Broderick et al. 2014).

14.3 Relationship Between Vertebrate and *Drosophila* Following the Gut Morphology

14.3.1 General Organization of the Digestive System of Mammals and *Drosophila*

The mammalian digestive system consists of a series of gastrointestinal tract (GI) organs, which forms the gut. Various compartments present in the gut are the esophagus, stomach, and small and large intestine (Fig. 14.1a). The food passes through different parts of the digestive system and mixes with enzymes secreted from the liver, pancreas, and intestine due to the involuntary action of muscles of the digestive system, and finally from the intestinal layer, they are absorbed (Thompson et al. 1983). The *Drosophila* gut shares its homology with the mammalian gut (Fig. 14.1b). Foregut, midgut, and hindgut are the three parts of the *Drosophila* gut. Foregut or stomodeum includes a buccal cavity, pharynx, esophagus, crop, and proventriculus or gizzard where the food is stored, ground, and transported to the midgut (Fig. 14.1b). The midgut of the mesenteron is the site where digestive enzymes get secreted into the lumen and thus helps in the absorption of nutrients from the food. The epithelial layer present in the peritrophic membrane and midgut is utilized in digesting and absorbing food particles (Edgecomb et al. 1994; Apidianakis and Rahme 2011). Water, minerals, and other beneficiary substances before excretion occur through proctodeum. The Malpighian tubule connects the hindgut and midgut and plays the main role in osmoregulation and excretion

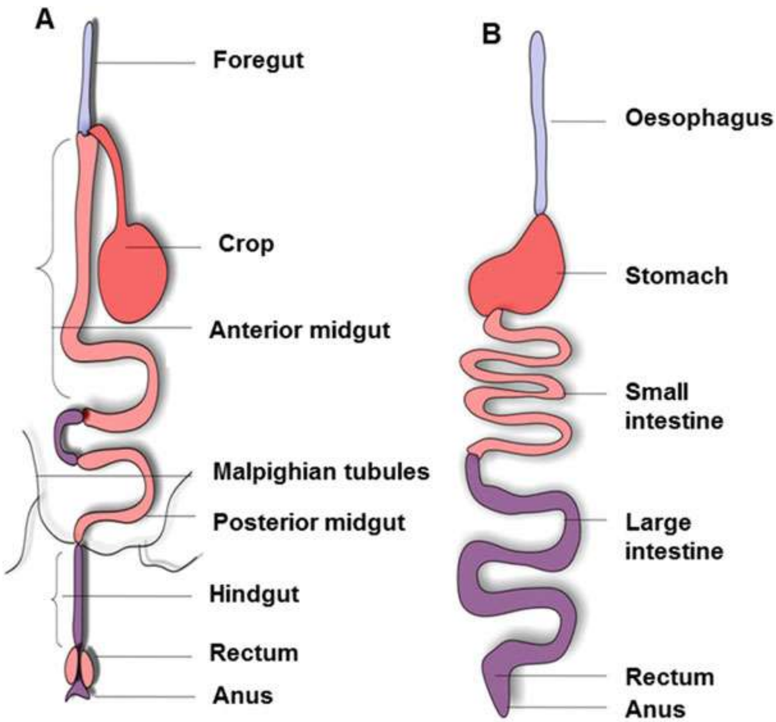


Fig. 14.1 Comparative structural difference between the gut of *Drosophila* and mammal. (**a**) The gut of *Drosophila* showing the foregut, midgut, hindgut regions, rectum, and anus. (**b**) Mammalian gut showing the esophagus, stomach, small intestine, large intestine, rectum, and anus

(Apidianakis and Rahme 2011). The midgut is considered to be the main site of digestion and is enriched with microbes (Neish 2009).

14.3.2 Anatomical Similarities and Dissimilarities Between *Drosophila* and Mammalian Intestine

Human and *Drosophila* intestine share similarities in terms of tissue structure, anatomy, physiology, and developmental point of view (Pitsouli et al. 2009; Rubin 2007). Fly and mammalian gut are originated from endothelial cells (Kedinger et al. 1987) and formed of a monolayer of columnar and cuboidal cell known as enterocytes (Fig. 14.2a). Crypts of Lieberkühn is a depression in the surface of the large and small intestine with a structure called villi to increase the surface area of absorption (Crosnier et al. 2006) (Fig. 14.2a, b). The microvilli of apical enterocytes and intestinal stem cells perform a similar function like vertebrates in the case of *Drosophila* (Shanbhag and Tripathi 2009) (Fig. 14.3a, b). Microvilli are parallel with each other and surrounded by a mucus layer that protects the host from

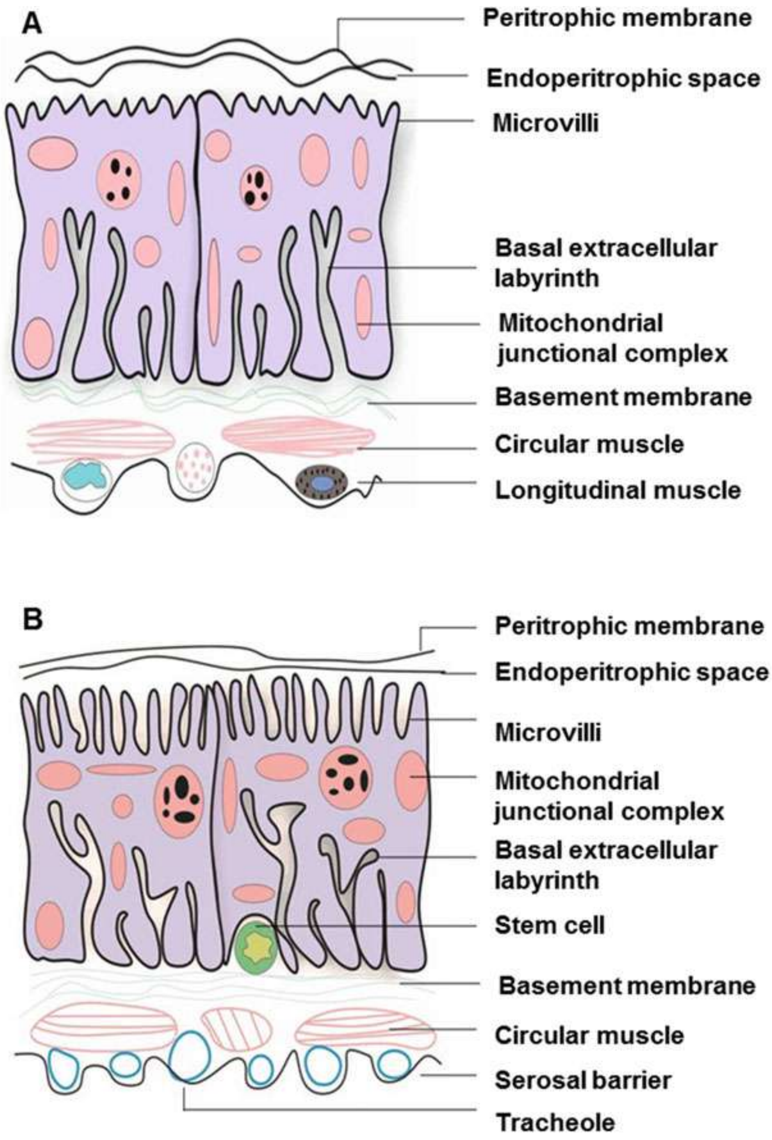


Fig. 14.2 Schematic representation of different regions of the midgut. (**a**) Anterior midgut. (**b**) Posterior midgut. The difference between the size of the microvilli of the anterior and posterior midgut has been shown

intestinal microbes (Fig. 14.2a, b). The mucous layer is acellular and comprising of chitin and glycoprotein such as peritrophic to prevent the microbe from invading the midgut and hindgut.

Mammals and fly on their basal side of the basement membrane contain epithelial monolayer. Longitudinal and circular muscles present below the basement

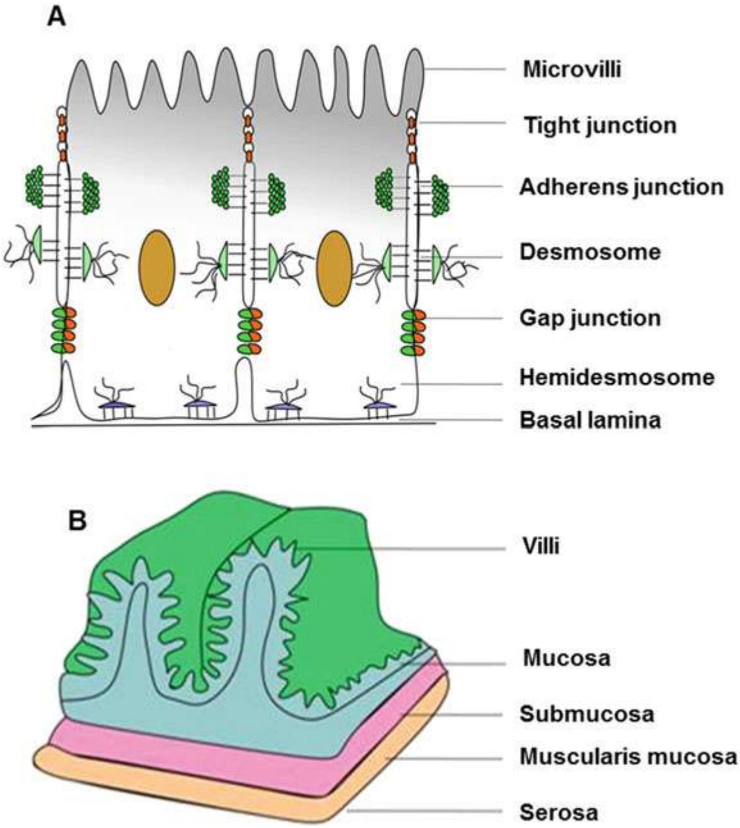


Fig. 14.3 Schematic representation depicting the internal organization of the gut. (**a**) The cuboidal epithelium of gut attached through different junctions like a tight junction, adherens junction, desmosome, and gap junction. (**b**) Various layers of gut epithelium are villi, mucosa, submucosa, muscular mucosa, and serosa

membrane improve peristalsis in *Drosophila* (Fig. 14.2a, b). Such muscular arrangement is also seen in mammals and helps in stimulation and oxygenation. The submucosa, muscular mucosa, and the lamina propria are present between the basement membrane and outer sub-muscle layer (Fig. 14.3b). The submucosa is a layer of densely populated connective tissues, lymphatic vessels, and blood vessels. Muscularis mucosa is another muscle layer. Peyer's patches, leukocytes, dendritic cells, mast cells, connective tissue, vessels, and micro-fibroblasts are present in lamina propria which is present below the intestinal epithelium. In *Drosophila* the midgut contains immune cells like enterocytes, secretory endocrine cells, and intestinal stem cells (Kuraishi et al. 2011).

14.4 The pH of *Drosophila* and Vertebrate Gut

Drosophila and mammalian digestion vary in terms of pH. In mammals, digestion occurs in an acidic condition, whereas *Drosophila* digestion occurs at neutral or basic pH (Apidianakis and Rahme 2011). In the larval gut, the anterior-most region is having pH 7, followed by an acidic region of pH 2, the next is a neutral region of pH 7, pH 6 is maintained in the next transition area, and the posterior-most area is alkaline in nature of pH 9.5 (Overend et al. 2016) (Fig. 14.4a). Adult *Drosophila* has a neutral pH in the anterior midgut region followed by a cupper cell region of pH 4. The posterior part of the midgut is less alkaline with a pH of about 7–9. The hindgut

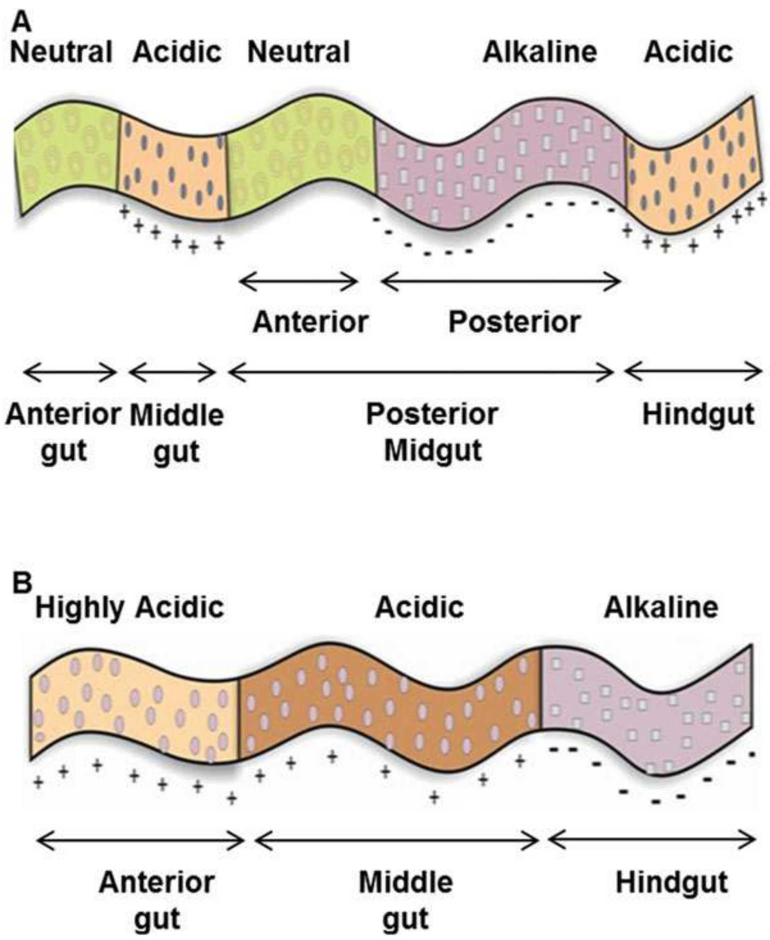


Fig. 14.4 The pictorial representation of different regions of the gut of *Drosophila* and humans according to pH. (**a**) The variation of *Drosophila* gut with different pH of neutral, acidic, neutral, alkaline, and acidic at a different level. (**b**) Different regions of the gut of mammals showing highly acidic, acidic, and alkaline

maintains pH 5. The acidic condition helps to protect the body from microbes and denatures proteins (Buchon et al. 2013a). In humans, the gut begins from the oral cavity with pH 6.7–7.3 (Baliga et al. 2013) followed by the esophagus with pH 5–7 then to intestines and finally to the anus (Guerra et al. 2012). The proteins and peptides get digested in the stomach with a highly acidic pH of 2 due to the secretion of hydrochloric acid. The pH variation of *Drosophila* and mammalian gut is depicted in Fig. 14.4b. All bacteria and pathogens get degraded here. Later the food moved to a less acidic environment of the small intestine, that is, 4–5 (McClements and Li 2010). All sorts of lipids, proteins, and fats get digested there. Finally, the large intestine maintains an alkaline medium. Besides pH, variation is observed in terms of enzymes in both *Drosophila* and vertebrate. The enzyme variation is summarized in Table 14.1. All the remaining foods that are not digested to small micro-molecules get degraded here (Tsabouri et al. 2014).

14.5 The Gut Bacterial Population of Mammals as well as *Drosophila*

The mammalian gastrointestinal tract is colonized with a few commensals. The gut contains 10–1000 numbers of bacteria belonging to five different phyla. Those include *Formicates*, *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, and Bacteroides such as *Staphylococcus*, *Veillonella*, *Streptococcus*, *Lactobacillus*, *Peptostreptococcus*, *Helicobacter*, and the *yeast Candida* (Simhadri et al. 2017). Among *Lactobacillus* nine different species are present in the gut like *L. plantarum*, *L. gasser*, *L. kalixensis*, *L. ultunesis*, *L. salivarius*, *L. fermentum*, *L. reuteri*, *L. antri*, and *L. gastricus* (Blum et al. 2013). Studies revealed that *Mycobacterium* mainly exists in the stomach in acidic pH (Thomson et al. 2007; Bodmer et al. 2000). In the intestine, two dominating phyla *Bacteroidetes* (gram-negative) and *Firmicutes* (gram-positive) are found. Basically in the small intestine, the microbes present such as *Proteobacteria*, *Verrucomicrobia*, *Tenericutes*, *Deferribacteres*, and *Fusobacteria* are found in the small intestine. It has been observed that the microbe population increases from the duodenum to the ileum. The duodenum contains 10^1–10^3, jejunum 10^4–10^7, and the ileum 10^{11}–10^{12} numbers of microbes (Neish 2009). Three factors, namely, the acidity of the stomach, pancreatic juice, and bile juice, all create a barrier to the growth of microbes. Microbes like *Enterococcus faecalis*, *Lactobacillus*, and *Diphtheroids* and the yeast *Candida albicans* are prevalent in the jejunum. In the ileum *Enterobacteriaceae* family have established their colony (Hayashi et al. 2005; Alp et al. 1999). Anaerobic, non-sporing, gram-negative, gram-positive, and spore-forming rods are among those microbes. Besides bacteria, certain yeasts (*Candida albicans*) (Kumamoto 2011) and protozoa (*Entamoeba hartmanni*, *Trichomonas hominis*, *Iodamoeba bütschlii*, *Endolimax nana*) are common inhabitants of the ileum. Bacteroides species like *Fusobacterium*, *Clostridium*, *Peptostreptococcus*, *Escherichia coli*, *Klebsiella*, *Proteus*, *Lactobacillus*, *Enterococci*, *Streptococci*, *Pseudomonas*, *Acinetobacter*, coagulase-negative

Table 14.1 Digestive enzymes of human being (Borgström et al. 1957; Whitcomb and Lowe 2007) and *Drosophila*

Carbohydrase		Proteinase		Lipase and esterase	
Drosophila	Human being	*Drosophila*	Human being	*Drosophila*	Human being
Amylase (3)	Salivary amylase	Aminopeptidase (12)	Pepsin	Lipase (29)	Pancreatic lipase
Mannosidase (7)	Pancreatic amylase	Carboxypeptidase (22)	Trypsin	Carboxylesterase (16)	Intestinal lipase
Glucosidase (10)	Maltase	Cysteine-type endopeptidase (9)	Chymotrypsin	Phospholipase (13)	
Trehalase (2)	Sucrase	Aspartic-type endopeptidase (12)	Dipeptidase	Sphingomyelin phosphodiesterase (3)	
Beta-galactosidase (2)	Lactase	Serine-type endopeptidase (132)	Carboxypeptidase	Phosphodiesterase (2)	
Lysozyme (15)	Dextrinase	Metalloendopeptidase (53)	Amino peptidase		
Chitinase (10)		Peptidyl dipeptidase (5)			
Amidase PGR (3)					

staphylococci, *Staphylococcus aureus*, *Mycobacterium*, and *Actinomyces* are also commonly found in the large intestine.

In comparison to vertebrate, very few species are reported from the *Drosophila* gut (Broderick and Lemaitre 2012). Two different phyla *Firmicutes* and *Proteobacteria* are majorly found in the gut. Among phylum *Firmicutes*, *Lactobacillus*, and *Enterococcaceae* families are found. Among *Proteobacteria* alpha and gamma classes of *Acetobacteraceae* and *Enterobacteriaceae* are known to be present in the gut. *Lactobacillus plantarum*, *Lactobacillus brevis*, *Enterobacter fecal*, and *Acetobacter pomorum* are the dominating microbiota within the gut. The commensal quantity varies from region to region and mostly affected by factors like environment, latitude, habitats, species, and laboratory conditions (Corby-Harris et al. 2007). Flies raised in laboratory conditions have a relatively low number of bacteria which includes genera like *Acetobacter* (*Acetobacter pomorum/ pasteurianus*) and *Lactobacillus* (*Lactobacillus plantarum*). Rare species like *Gluconobacter morbifer*, *Enterococcus fecal*, and *Enterobacteriaceae* group orbus are also found in flies raised in laboratory conditions. The lower bacterial population of the fly gut in comparison to the vertebrate could be due to the higher adaptive immune system in metazoan, short life cycle, and holometabolic type of development of *Drosophila* (McFall-Ngai 2007). To study the role of individual bacteria, flies were treated with 50 μg/mL of tetracycline for three generations and produce a germ-free fly (Sabat et al. 2015). These germ-free flies can be used to check the role of various microbes in the gut and thus currently used to study various host-microbe interaction.

14.6 Why Is It Essential to Have Bacteria in the Gut?

The metabolic, defense, and trophic activity of the body is maintained by gut microbes. The microbiota of the gut possesses certain barrier effects by checking pathogens to invade into intestinal mucosa so known to be the central line of the defense system. The mechanism is also called as colonization resistance. The microbes act via two different mechanisms: (1) by preventing the attachment of newly entered microbes with the epithelial cells of the gut and (2) by competing for available nutrients in ecological niches which offers a small microenvironment (Liévin-Le Moal and Servin 2014). These pathways help to protect the gut from the attack of foreign pathogens (Hooper et al. 2001). Besides protection, the microbiota further helps in the production of vitamins (vitamin K, folate, B12, and biotin), glycans, amino acids, xenobiotic, and SCFA. The microbes of the intestine guard epithelial barrier, which contributes to the regulation of the immune defense mechanism against the pathogens (Liévin-Le Moal and Servin 2014). Intestinal bacteria like *Bacteroides*, *Eubacterium*, *Propionibacterium*, and *Fusobacterium* are involved in the production of vitamins. Thus, microbiota present at the mucosal interface helps to shape a competent immune system. The gut-associated lymphoid tissue (GALT) arrangement is also affected by microbes of the GI tract (MacDonald and Monteleone 2005). GALT microflora helps in the development of systemic immune regulatory circuits and complex mucosa in initial developmental stages.

14.7 Gut Microbes as a Lifeguard Against Pathogens

Most organisms design several defense mechanisms and barriers to prevent the entry of pathogens to the gut. Still some microbes, for example, *Mycobacterium*, manage to escape into the GI tract of the gut. Once the microbes entered the GI tract, antagonistic activity of the pathogenic microbes over the commensals begins for site specificity and thus leads to infection or inflammation leading to severe diseases. There exists a common mechanism between human and *Drosophila* in terms of protection against pathogens. The defense mechanism against pathogens occurs in four different ways:

1. Physical barrier against the pathogens
2. Production of ROS
3. Secretion of antimicrobial peptides
4. Maintenance and renewal of epithelium in response to gut damage

14.7.1 Physical Barrier Against Pathogen

The physical barrier of humans shares homology with the *Drosophila*. In case of vertebrates, the gastrointestinal epithelium consists of various types of cells such as absorptive epithelial cells that enter endocrine cells (EEC), goblet cells, microfold cells, and Paneth cell. Out of all the cells, the absorptive cells (enterocytes) primarily function as a physical barrier against invading pathogens (Thirabunyanon 2011). Desmosomes, adherent junctions (AJs), tight junctions (TJs), ions, and mucus are secreted from microvilli, a modified form of the apical lining of the intestinal epithelium. These junctions act as a physical barrier against pathogens and thus help to maintain the integrity of the human gastrointestinal tract. TJs and AJs further help to maintain the polarity of the cell (Fig. 14.4b). The EEC acts as a sensor and forms the first-line control of the brain-gut axis (Moran et al. 2008). Mucin which is the basic component of mucus is secreted by the goblet cells. Microfold cells as well as Paneth cells also act as a barrier and defend the gut from an enteric pathogen (Bevins and Salzman 2011). Nucleotide-binding oligomerization domain protein and Toll-like receptors can sense the microenvironment of the intestinal epithelium and trigger chemokines and cytokines that signal for the stimulation of leukocytes against inflammatory response (Thirabunyanon 2011).

The peritrophic membrane, the analogous structure of the mucus membrane of the mammalian digestive tract, acts as a barrier and helps to protect the midgut from xenobiotics and toxins. *Drosophila* peritrophic membrane encodes chitin-binding protein drosocrystallin (Dcy). Any mutation or drugs may decrease the width of the matrix and thus can increase its permeability (Kuraishi et al. 2013a) which is regulated by protein-protein cross-linking (Shibata et al. 2013).

14.7.2 Formation of Reactive Oxygen Species (ROS)

Upon bacterial infection immune system provokes an initial response in the form of ROS. ROS is highly toxic to the microorganism and thus prevents the microorganism to colonize within the host. ROS interacts with the host's signaling pathways and thus eliminates the microorganism in vertebrates (Spooner and Yilmaz 2011). A similar mechanism is also reported in *Drosophila*. In *Drosophila*, ROS is produced only with heavy infection to eliminate pathogens (Kuraishi et al. 2013a). ROS produced by *Drosophila* gut is uracil mediated and DUOX dependent (Lee et al. 2013). In the plasma membrane, enzyme DUOX is present, which is a family member of NADPH oxidase. NADPH oxidase destroys engulfed bacteria both in phagosomes and in neutrophils (Babior 1999). Pathogenic bacteria *E. carotovora* and *Gluconobacter morbifer* release uracil that activate*s* the production of g-coupled receptors resulting in activation of DUOX and NADPH oxidase enzyme. A G-protein mobilizing Ca^{2+} production leads to the activation of uracil phospholipase Cβ (PLCβ) enzyme in gut epithelial cells. Proliferation and maturation of ISCs are the results of excess ROS. The high amount of ROS causes cytotoxicity within the gut.

14.7.3 Secretion of Antimicrobial Peptides (AMPs)

To protect the host from pathogens, host cells secrete certain evolutionarily conserved peptides called antimicrobial peptides (AMPs) (Zasloff 2002). The AMPs are formed from 20 to 40 amino acids and are of two types: defensins and cathelicidins. The Paneth cells of the crypts of Lieberkühn secrete the AMPs (Nicolas and Liévin-Le Moal 2015). In humans, probiotics produce AMP which has antagonistic activity against intestinal pathogens. AMP can accelerate the chlorine concentration and thus manipulate the microbial population in culture. Ideal physiological conditions may permit AMPs to initiate chemical defense by providing innate immunity against crypt microenvironment. Bacteriocins are a good antimicrobial peptide of ribosomal origin and are effective against the gastrointestinal pathogen (Thirabunyanon 2011; Kopp et al. 2015).

In *Drosophila* during systemic infection, the fat body produces AMPs (Lemaitre and Hoffmann 2007). There are two ways by which AMPs are produced, that is, Toll and IMD pathways. But in the gut, the IMD pathway mainly generates AMPs. Gram-positive bacteria contain peptidoglycan recognition protein (PGRP) LE and PGRPLC, while gram-negative bacteria express the diaminopimelic acid on its surface which is recognized by receptors present in the host (Bosco-Drayon et al. 2012; Neyen et al. 2012). The infection of *E. carotovora* or *P. entomophila* resulted in the activation of IMD pathways. IMD mutant flies show more oral defects than normal ones. The infection of IMD flies with *P. entomophila* and *S. marcescens* proves that this pathway is responsible for gut defects (Liehl et al. 2006; Nehme et al. 2007). Often pathogen possesses some gene to overcome the activity of AMP. For instance, *P. entomophila* contains a gene *arpA* for improving its virulence by encoding one enzyme metalloprotease that defends the microbe in opposition to AMPs by neutralizing it. Bacteria use such an approach for fighting with the immune system of the gut.

14.7.4 Maintenance and Renewal of Damage

Both vertebrate and *Drosophila* gut may get damaged due to bacterial infection. The gut recovery is possible if the immune response acts in coordination with epithelial renewal. The ability to sustain an infection depends on the ability to resist, to eliminate the pathogen, and also to tolerate, which increases the host capacity against infection. Bacteria damage the gut either by bacterial toxins against the epithelium and over the activity of the immune system such as the ROS pathway. The old or damaged enterocytes are replaced with the newly produced ISCs that differentiate into enterocytes. The JAK-STAT and Egfr signaling play a key role to regulate these mechanisms. The JAK-STAT ligands Upd2 and Upd3 can trigger the mechanism. The Upd3 induction is done by triggering both JNK and Hippo pathways (Buchon et al. 2013b; Panayidou and Apidianakis 2013).

An array of both innate and adaptive immune responses is evoked by the immunomodulation when immune cells interacted with probiotics. This immunomodulation is carried out by the intestinal stem cells (ISCs) of the human intestinal epithelium (Thirabunyanon 2011). The oligomers of endoplasmic reticulum produce mucins that bind to ligands of pathogenic surfaces. Several divisions of mucins are gel-forming, membrane-bound, and soluble mucin and are of two different groups: gel-forming and non-gel-forming (Gouyer et al. 2015). Mucin plays a significant role in fetal development, epithelial renewal, carcinogenesis, and metastasis along with protection and lubrication of the epithelial lining. The mucosal gel is useful for both the normal community and the pathogenic microbiota in providing energy sources from oligosaccharides and in cryoprotection (Liévin-Le Moal et al. 2011).

For maintenance of homeostasis in *Drosophila*, the proliferation of ISCs is an important factor. This step is regulated by Delta-Notch, Wingless, and Pvr signaling pathways. In mammals, the proliferation of ISCs is regulated by signaling pathways (wnt, Hedgehog, and BMP) and some transcription factors (CDX1, CDX2, and HNF1) (Thirabunyanon 2011; Kuraishi et al. 2013b). Cytokines help in the proper management of the immune system are produced by the tumor necrosis factor (TNF), interferons (IFN), and interleukins (IL). TNF, IL-1, and IL-6 act like growth factors during gastric carcinoma and hematological malignancies. Cell death suppression, stem cell activation, and proliferations are also done by them at the site of injury. In both mammals and fruit fly, the signaling mechanisms to maintain ISCs are mediated by the cytokines during regenerative inflammation and cancer. The ISCs of both humans and fly play a pivotal role in maintaining intestinal cells and thus gut homeostasis by various conserved signaling pathways.

14.8 Conserved Signaling Pathways Associated with the Microbe Infection

14.8.1 Wnt/Wg Pathway

This pathway involves two different proteins that interact with 20 different receptors. Wnt signaling is transduced by either β-catenin or TCF4. Usually, near the ISCs which are present in the intestinal epithelium, Wnt signaling is activated and helps in proliferation of ISCs so that it regulates various phenomena such as cell fate determination and stem or progenitor cell maintenance in vertebrates (Nicolas and Liévin-Le Moal 2015). Whenever the gradient of Wnt decreases, differentiation of ISC occurs. This pathway is associated with the significant generation of many developmental gradients in response to a wingless ligand for Wnt (Buchon et al. 2013b). Contrary to it is the Wg pathway which pivots the ISC proliferation in *Drosophila***.** Increased mitosis of ISC shares similarity with mammals due to over-activation of the Wg pathway. Mutation in the APC gene in mammals leads to intestinal cancer. A homologous in the APC gene mutation in *Drosophila* behaves as antagonist to Wg mechanism (Apidianakis and Rahme 2011).

14.8.2 Toll Pathway/Toll-Like Receptor Pathway

Toll pathway is associated with fungal infection in *Drosophila*. It is like an immune sensor and sometimes regulates fat body metabolism and hemocyte numbers (Christofi and Apidianakis 2013; Kuraishi et al. 2013b). Hemolymph activates the Toll pathway by a serine protease which involves neuroserpin and processes the putative Toll ligand spätzle. Once Toll binds to the spätzle, it activates intercellular signaling cascades that involve dMYD88, tube, and the kinase Pelle (Panayidou and Apidianakis 2013). This helps in the degradation of cactus (an IK-b like protein) and translocation of NFKB and dorsal. In gram-positive bacteria, because of the recognition of peptidoglycan by peptidoglycan recognition protein (PGRP-SA), the Toll pathway is triggered. This pathway is activated by soluble recognition molecules triggering proteolytic activity converting to spätzle (De Gregorio et al. 2002). In the case of mammals, the toll-like pathway is activated by the direct association of the receptors with the pathogens. In *Drosophila*, in the case of tube and Pelle which are utilized in mammalian TLR signaling, IRKA4 and IRKA1 are used. Similarly, TAK1/TAB is activated by IRAKs and TRAF6 recruited by MYD88 (Panayidou and Apidianakis 2013).

14.8.3 Imd Pathway/TNFR Signaling Pathways

The gram-negative bacteria activate the IMD pathway in *Drosophila*. Cytotoxins and diaminopimelic acid peptidoglycan (DAP-PGN) are recognized by the *intracellular* receptor PGRP-LE and membrane receptor PGRP-LC, respectively, and receptors get activated. This signal mechanism will induce IMD expression leading to increased translocation of Relish. Relish with NF-κB boosts the antimicrobial peptide expression and negative regulation of this pathway. TCT and peptidoglycans are destroyed by the catalytic activity of PGRP-LB and PGRP-LC. This hampers the ligand recognition capacity of receptors such as PGRP-LF, PGRP-LC, and PGRP-LE by decreasing the concentration of ligands. Transcription response of Relish is controlled by caudal and nuclear translocation of Relish which is blocked by transglutaminase. The tumor necrosis factor receptor signaling pathway is similar to the Imd pathway that regulates NFKB which indicates an evolutionary link between fly and mammals (De Gregorio et al. 2002).

14.8.4 Notch Signaling Pathway

The Notch signaling promotes differentiation or proliferation of ISC in *Drosophila melanogaster*. This pathway induces ISC, intestinal development, and the differentiation of hair follicles in humans (Apidianakis and Rahme 2011; Nicolas and Liévin-Le Moal 2015). However, there exists a similairty between *Drosophila* and mammalian Notch signaling when enterocyte is the fate of an intestinal cell in secretion (Apidianakis and Rahme 2011). During Notch signaling, the enteroendocrine cell or the enterocyte is differentiated from the enteroblast cells (Panayidou and Apidianakis 2013).

14.8.5 JAK-STAT Pathway

This pathway gets activated upon infection with gram-negative bacteria like *Erwinia carotovora*, *Serratia marcescens*, or *Pseudomonas entomophila* (Apidianakis and Rahme 2011). The intestinal enterocytes produce secretory cytokine, Upd1, Upd2, and Upd3 upon infection which leads to ISC divisions. Mammalian cytokine IL6 stimulates cancer progression as well as inflammation of this pathway. Also, this provides information regarding the mechanism of intestinal regeneration and homeostasis (Apidianakis and Rahme 2011; Kuraishi et al. 2013b). JAK-STAT pathway antagonizes Notch signaling pathway while determining the fate of enteroblast (Panayidou and Apidianakis 2013).

14.8.6 NF-κB Signaling Pathway

During the attack of microbes, the nuclear factor-kB plays a crucial role (Rahman and McFadden 2011) in response to intestinal injury in *Drosophila* when the induction of the Imd pathway is inhibited to avoid harmful effects due to long-lasting initiation of the immune system. A similar mechanism is seen in the human intestine for managing gut contamination by this localized response to peptidoglycan PGRP-LE and PGRP-LC which activate NF-κB. The Imd pathway is hampered by PGRP-LE (Kuraishi et al. 2013a).

14.9 Gut Bacteria in the Treatment of Disease (Probiotics)

Initially, it was known that a substance which is a product of microbes and can be useful in mediating the growth of another organism acts as a "probiotics." Later this probiotics definition changes in a time-dependent manner. The current definition of probiotics is termed by Marteau et al. (2004) which means one microbial community has a beneficial impact on others (Marteau et al. 2004). In a healthy human being, 90% of gut microbiota are probiotics. This microenvironment can be helpful in extracting energy from food and enhance immunity in the body by inhibiting obesity and infection. The good microbial fauna can be disturbed largely by antibiotics, antacids, excessive alcohol, sugar, NSAIO's radiation, chlorine and fluorine, bacterial dysentery, and high meat, fat, and sugar diets. Besides these factors, a pathogenic bacterium invades the GI tract and multiples, leading to the loss of good bacteria. Probiotics help to maintain the homeostasis by maintaining growth and regeneration, maintaining pH, regulating the digestive and immune system, and protecting from antitumor potentials. Choosing a probiotic bacteria should have several criteria as follows: (a) it must be of human origin with non-pathogenic behavior; (b) it can resist technological pressure, gastric acidity, and bile of GI tract; (c) it is efficient to adhere to the gut epithelium; (d) it should produce antimicrobial substance; (e) it should have the capability to modulate immune response; and (f) it should offer incentive for metabolism (e.g., production of enzymes, absorption of cholesterol, and maintaining actions of lactose). According to a well-designed double-blind, placebo-controlled (DBPC), human studies, probiotics can be selected. Two mechanisms are established for the proper functional efficiency of probiotics.

1. The ability of the microflora to stay inside the host environment and form colonies within the host environment. The probiotics act on the intestinal epithelium by (1) synthesizing antimicrobial peptides, (2) competitive exclusion, (3) testing for nutritional products, (4) improving intestinal barrier operation, and (5) immunomodulation.
2. The ability of the host to maintain the colonies (Erkosar et al. 2013).

Combined probiotics are proved to be more efficient than single probiotics in terms of targeting specificity associated with the human intestine along with the commensals (Table 14.2). The probiotics eliminate pathogens adhering to the

Table 14.2 List of probiotics used either individually or in various combinations to treat various diseases

Probiotics	Used for treatment	Reference
Lactobacillus rhamnosus, *Lactobacillus casei Shirota*, *Lactobacillus reuteri*, *B. lactis, Bb-12*	Diarrhea	Salminen et al. (2010)
Lactobacillus casei DN114001	Respiratory disease	Salminen et al. (2010)
Lactobacillus acidophilus NFCM with *Bifidobacterium animalis*	Immunization against cold and influenza	Salminen et al. (2010)
Strain of *Lactobacillus*	Mycotoxin (aflatoxin B1), cyanobacterial toxins, hepatotoxin, microcystin, and nodularin	Salminen et al. (2010)
L. johnsonii ncc533, *L. casei Shirota*, *L. acidophilus LB*	*Rotavirus* and *Helicobacter pylori* which cause gastric ulcer and cancer	Thirabunyanon 2011, Liévin-Le Moal and Servin (2014)
Lactobacillus casei DN-114001	The HT-29 MTX cell infection by RF and WA strains of rotavirus can be inhibited by this microbe	Varyukhina et al. (2012)
Lactobacillus rhamnosus	Against antibiotic-associated diarrhea due to *C. difficile*	Thirabunyanon (2011)
Enterococcus faecium PCD71 and *Lactobacillus fermentum* ACADC179	*Salmonella enteritidis* contamination by inhibiting its growth	Thirabunyanon (2011)
Bifidobacterium longum Bar33, *Lactobacillus plantarum* Bar10, *Lactobacillus acidophilus* Bar13, and *B. lactis* Bar30 strains	The epithelial cell infection is caused by *S. typhimurium* in the intestine	Thirabunyanon (2011)
Lactobacillus casei Shirota YIT9029, *Lactobacillus salivarius* UCC118, *Lactobacillus plantarum*, *Lactobacillus delbrueckii* var. bulgaricus UFV-H2620	Growth of *L. monocytogenes*, *L. acidophilus* PY2 is affected	Thirabunyanon (2011)
Enterococcus faecium, *Bifidobacterium lactis* 420, *Lactobacillus plantarum* 299v	Bactericidal to strains of *E. coli*	Thirabunyanon (2011)
Lactobacillus helveticus and *Bifidobacterium longum*	Anxiety decreased and psychological behavior	Cryan and Dinan (2012)
Lactobacillus plantarum	Produces pro- and anti-inflammatory cytokines along with blood mononuclear cells	Thirabunyanon (2011)

epithelial cell surface by competitive exclusion (Thirabunyanon and Hongwittayakorn 2013). The combination may be counteractive if not selected according to preclinical setups.

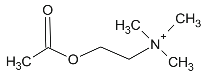
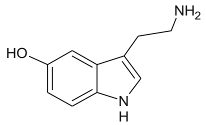
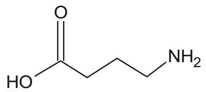
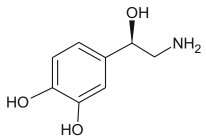
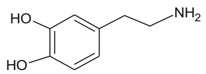
14.10 Role of Gut Microbes Beyond the Gut

The weight of the bacteria present in our gut weighs equal to the weight of our brain. A recent study reveals gut microbiota's role in the development of the brain and social interaction of mammals. Almost 150–200 bacteria are commonly found, and around a thousand less frequently found bacteria are within the gut. The microbiome is the microenvironment of the human gut, and the human genome is less diversified than the genes of microbes (Hamady and Knight 2009). In metagenomic technologies, a study of the composition of gut microbe from early developmental stages (Palmer et al. 2007) to the elder stage (Claesson et al. 2012) is done. The most physiological effect of microbes on the health of the host is not clearly understood. The human brain is not fully developed during early development, and the gut is even sterile. Sterile womb prototype shows challenges to microbes already present in the uterus (Funkhouser and Bordenstein 2013). During normal delivery, the baby passes through the birth canal and thus exposed to the mother's microbiota. In a C-section baby exposed to the microbes of the hospital environment, microbe's colonization occurs in the body. Brain development requires the colonization of microbes during early post-natal weeks.

How microbes govern the brain behavior as well as the development of organisms seems to be a challenging question at this moment. Gut microbes live in a symbiotic relationship, get the nutrition from the human body, and help in the development and functioning of the brain (Geurts et al. 2014; Chen et al. 2013; Ranjan et al. 2018a,b). It can produce a large number of neuroactive compounds or neurochemicals essential for our brain development (Table 14.3). Thus the absence of microbes greatly alters brain neurochemistry. The brain's serotonergic system, which regulates the emotional activity, requires a microbe for its development (Clarke et al. 2013). Because of the diversity of microbes in the gut, the human brain evolved (Montiel-Castro et al. 2013). In an experimental study of rodents raised in microbes, free condition exhibited an alteration in sociability with a clear autistic defect in their behavior (Desbonnet et al. 2014). Because of the gene transfer by bacteria, and its descendants, many human genes show similarities with that of bacterial genes (McFall-Ngai et al. 2013). The microbe colonization in the gut also improves the living conditions of other bacteria in the gut (Lombardo 2008; Troyer 1984). Also, the basic behavioral patterns such as cognition are regulated by gut microbes.

The brain-gut-microbiota (BGM) axis shows the relationship of gut microbes with the brain. The microbes usually interact with the autonomic, central, and enteric nervous system along with the immune and endocrine system via hypothalamic-pituitary-adrenal axis (HPA axis) for a complete series of coordination of the gut-brain axis. Parasympathetic and sympathetic branches are also involved (Mayer 2011). The network extends to smooth muscles and the central nervous

Table 14.3 List of bacteria, specific neurotransmitters secreted by them, and their role in the physiological system

Bacteria	Neurotransmitter	Structure	Function
Lactobacillus	Acetylcholine		Responsible for the stimulation of muscles (gastrointestinal system)
			Has a part in scheduling REM (dream) sleep
Bifidobacterium infantis, *Candida*, *Streptococcus*, *Escherichia* and *Enterococcus* sp.	Serotonin		Serotonin depletion will generate depression, so they are helpful in determining mood behaviors
Lactobacillus rhamnosus and *Bifidobacterium dentium*	GABA		Responsible for calming our nervous system and reducing anxiety and mental ruminations often seen with depression and OCD
Escherichia, *Bacillus*, and *Saccharomyces* sp.	Norepinephrine (stress hormone)		Responsible for vigilant concentration, cognitive alertness
			Medically used in case of severing hypotension
Bacillus	Dopamine		Role in reward-motivated behavior
			Acts as a vasodilator and inhibits norepinephrine release
			Reduces GI motility and protects intestinal mucosa

system by afferent and efferent fibers, respectively (Grenham et al. 2011). Thus, the brain induces signals to travel through the nervous system by motor neurons to sensory neurons, and the gut, in contrast, maintains brain function by sending its signal to the brain axis by such a two-way mechanism. Cranial nerve X is the main mediator of such signaling (Dinan and Cryan 2013). For increasing probiotic development in the gut as well as proper brain activity, vagus nerve activation is important. Bacteria transduces its effect on CNS via the immune system in a bidirectional way (Stilling et al. 2014; Hueston and Deak 2014). The innate immune system is also affected by the gut microbes by changing the concentration of circulating anti- and pro-inflammatory cytokines that also affect brain functioning. The hypothalamus is the master controller of all the events going on in the brain relating to hormone release also. Among such functions, one of them is the release of corticotrophin-releasing hormone (CRH) by the stimulating effect of Interleukin-6 and Interleukin-1 from the anterior pituitary.

By producing short-chain fatty acid (SCFAs), bile, and other metabolites like choline, gut microbiota can influence neuron expression. SCFAs like n-butyrate, acetate, and propionate have neuroactive properties. Neurotransmitters are released by microbes residing in the lumen of the intestine so that it can catalyze several signaling pathways in the efferent nervous system by the release of modulators by the epithelial cells or operate straight at afferent axons. Among all the probiotics, *Bifidobacterium infants* are used to treat several neuronal diseases like depression and brain diseases. In a rat model, the *Bifidobacterium infantis* 35624 increases the tryptophan level. For the neurotransmitter serotonin, tryptophan acts as a precursor. Usually, kynurenine (a metabolic product of peripheral tryptophan) can be altered by *Bifidobacterium infantis*. Cognition is altered in neurodegenerative diseases like Alzheimer's and multi-infarct dementia at the age of 85. A direct correlation between gut microbes and cognition is not yet established. However, the lowest diversity of gut microbiota is reported from the elderly and those with cognitive dysfunction.

14.11 Gut Microbes Regulating the Physiology and Behavior

Modern studies highlighted the prominence of gut microbiota on the mate selection process and thus the evolution of new species. Modification in gut microbiota can be due to the social interaction of an organism (Koch and Schmid-Hempel 2011). Scent gland modification is known to be carried out due to change in composition in gut microbiota which leads to variation in kin recognition as well as sex determination. The role of gut microbes in the sex determination of *Drosophila* is established by Lize et al. In children, gut microbes are further known to be associated with autism spectrum disorder (ASD). Gut microbes regulate circadian rhythm and thus the metabolic activity of the body. A disease like diet-induced obesity resulted due to an imbalance in gut bacteria (Leone et al. 2015). Gut microbes are thought to affect the developmental stages in mice; reduction of anxiety is also carried out by them which further leads to changes in the behavior of mice (Heijtz et al. 2011). Diet and exercise together can change the body weight, anxiety, and cognitive behavior of the animal (Kang et al. 2013).

Stress can affect the health of human beings in various ways (positive or negative). When it affects in a positive way, it is called a pivot point (Yerkes and Dodson 1908), and beyond this, it can hazard the health and life of an organism (Bregman and McAllister 1983). Consumption of probiotics can change the normal microflora and subsequently affect the processing of information in the emotional material, which is established by the functional magnetic resonance imaging (fMRI) study. Urinary free cortisol (UFC) amount can be declined by combined treatment of *Bifidobacterium long* R0175 and *Lactobacillus helveticus* R0052 for 30 days (Messaoudi et al. 2011). Psychobiotics can modify mental health processes and manage stress. All the information in combination specifies that complex gut microbiota may regulate the neuronal function, which impacts health and disease when absent.

14.12 *Drosophila* as a Model to Screen Probiotics

Probiotics as an alternative to antibiotics have proved to be promising in various fields starting from pisciculture to treat the neurodegenerative disorder. Thus, various labs and pharma companies are in the process of isolating probiotics, and a model organism is required to screen the probiotics. Oral administration of probiotics is the most appropriate route as it will deliver the bacteria directly to the target site. Feeding of probiotics to *Drosophila* mimics mammalian intestinal infection. Microbes will be introduced into the gut via feeding, and thus various signaling pathways associated with intestinal regeneration will be active. These signaling pathways are conserved between vertebrate and *Drosophila*. This advocates that *Drosophila* can be used as a good model to check the activity of probiotics. Furthermore, probiotics can be tagged with a fluorophore. Fluorophore tagged with probiotics will be administered orally, and thus the localization and population within the gut can be checked easily under a fluorescence microscope. Probiotics like *L. plantarum* against microbe-induced gut damage during infection was analyzed in *Drosophila* (Phumkhachorn et al. 2007). Due to a shorter life cycle and with all available tools, the effect on neurons can be easily analyzed by various behavioral studies. *Drosophila* neurons are more susceptible to stress, and thus any change caused by the microbes can be reflected in the form of a behavioral phenotype. Fly behavioral phenotype can be easily monitored via various cost-effective methods like geostrophic assay, flight test, courtship test, etc. (Nichols et al. 2012). If probiotics are affecting the neurotransmitters, then it will be reflected in the walking behavior, growth, and development of flies. Thus the probiotics screening can be studied using *Drosophila* as a model organism.

14.13 Conclusions

Drosophila gut shares a variety of structural, developmental, and functional similarities with the vertebrate gut, so it can be effectively employed as a model organism to understand the relationship between the gut and microbe. Various laboratories are busy in isolation of probiotics as an alternative approach to antibiotics for treating various diseases. Isolation of a large number of probiotics needs a screen by using an in vivo model. The functional aspects of probiotics can be studied with the help of *Drosophila* as various signaling pathways involved during the microbe's infection remain conserved between *Drosophila* and human being. More importantly, since microbes regulate the mode of action of neurons, thus the behavior of the whole organism and mode of action of microbes can be scored on that basis.

Acknowledgments S. Sahu is grateful to the DST-Inspire fellowship for financial support. MM lab is supported by Grant No. BT/PR21857/NNT/28/1238/2017, EMR/2017/003054, Odisha DBT 3325/ST (BIO)-02/2017.

References

Alp M, Kocabağli N, Kahraman R, Bostan K (1999) Effects of dietary supplementation with organic acids and zinc bacitracin on ileal microflora, pH and performance in broilers. Turk J Vet Anim Sci 23(5):451–456

Apidianakis Y, Rahme LG (2011) *Drosophila melanogaster* as a model for human intestinal infection and pathology. Dis Model Mech 4(1):21–30

Babior BM (1999) NADPH oxidase: an update. Blood 93(5):1464–1476

Baliga S, Muglikar S, Kale R (2013) Salivary pH: A diagnostic biomarker. J Indian Soc Periodontol 17(4):461

Bevins CL, Salzman NH (2011) Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. Nat Rev Microbiol 9(5):356–368

Blum JE, Fischer CN, Miles J, Handelsman J (2013) Frequent replenishment sustains the beneficial microbiome of Drosophila melanogaster. MBio 4(6):e00860–e00813

Bodmer T, Miltner E, Bermudez LE (2000) Mycobacterium avium resists exposure to the acidic conditions of the stomach. FEMS Microbiol Lett 182(1):45–49

Borgström B, Dahlqvist A, Lundh G, Sjövall J (1957) Studies of intestinal digestion and absorption in the human. J Clin Investig 36:1521

Bosco-Drayon V, Poidevin M, Boneca IG, Narbonne-Reveau K, Royet J, Charroux B (2012) Peptidoglycan sensing by the receptor PGRP-LE in the *Drosophila* gut induces immune responses to infectious bacteria and tolerance to microbiota. Cell Host Microbe 12(2):153–165

Bregman NJ, McAllister HA (1983) Constraints on the Yerkes-Dodson law in skin temperature biofeedback. Int J Neurosci 21(3-4):183–189

Broderick NA, Lemaitre B (2012) Gut-associated microbes of *Drosophila melanogaster*. Gut Microbes 3(4):307–321

Broderick NA, Buchon N, Lemaitre B (2014) Microbiota-induced changes in *Drosophila melanogaster* host gene expression and gut morphology. mBio 5(3):e01117–e01114

Buchon N, Broderick NA, Lemaitre B (2013a) Gut homeostasis in a microbial world: insights from *Drosophila melanogaster*. Nat Rev Microbiol 11(9):615–626

Buchon N, Osman D, David FP, Fang HY, Boquete J-P, Deplancke B, Lemaitre B (2013b) Morphological and molecular characterization of adult midgut compartmentalization in *Drosophila*. Cell Rep 3(5):1725–1738

Chandler JA, Lang JM, Bhatnagar S, Eisen JA, Kopp A (2011) Bacterial communities of diverse *Drosophila* species: ecological context of a host–microbe model system. PLoS Genet 7(9): e1002272

Chen X, D'Souza R, Hong S-T (2013) The role of gut microbiota in the gut-brain axis: current challenges and perspectives. Protein Cell 4(6):403–414

Christofi T, Apidianakis Y (2013) *Drosophila* immune priming against Pseudomonas aeruginosa is short-lasting and depends on cellular and humoral immunity. F1000Res 2:76

Claesson MJ, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, Harris HM, Coakley M, Lakshminarayanan B, O'Sullivan O (2012) Gut microbiota composition correlates with diet and health in the elderly. Nature 488(7410):178–184

Clarke G, Grenham S, Scully P, Fitzgerald P, Moloney R, Shanahan F, Dinan T, Cryan J (2013) The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. Mol Psychiatry 18(6):666–673

Corby-Harris V, Pontaroli AC, Shimkets LJ, Bennetzen JL, Habel KE, Promislow DE (2007) Geographical distribution and diversity of bacteria associated with natural populations of *Drosophila melanogaster*. Appl Environ Microbiol 73(11):3470–3479

Crosnier C, Stamataki D, Lewis J (2006) Organizing cell renewal in the intestine: stem cells, signals and combinatorial control. Nat Rev Genet 7(5):349–359

Cryan JF, Dinan TG (2012) Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat Rev Neurosci 13:701–712

Cummings J, Macfarlane G (1997) Role of intestinal bacteria in nutrient metabolism. Clin Nutr 16(1):3–11

De Gregorio E, Spellman PT, Tzou P, Rubin GM, Lemaitre B (2002) The Toll and Imd pathways are the major regulators of the immune response in *Drosophila*. EMBO J 21(11):2568–2579

Desbonnet L, Clarke G, Shanahan F, Dinan T, Cryan J (2014) Microbiota is essential for social development in the mouse. Mol Psychiatry 19(2):146

Dinan TG, Cryan JF (2013) Melancholic microbes: a link between gut microbiota and depression? J Neurogastroenterol Motil 25(9):713–719

Edgecomb RS, Harth CE, Schneiderman AM (1994) Regulation of feeding behavior in adult *Drosophila melanogaster* varies with feeding regime and nutritional state. J Exp Biol 197(1): 215–235

Engel P, Moran NA (2013) The gut microbiota of insects–diversity in structure and function. FEMS Microbiol Rev 37(5):699–735

Erkosar B, Storelli G, Defaye A, Leulier F (2013) Host-intestinal microbiota mutualism: "learning on the fly". Cell Host Microbe 13(1):8–14

Funkhouser LJ, Bordenstein SR (2013) Mom knows best: the universality of maternal microbial transmission. PLoS Biol 11(8):e1001631

Geurts L, Neyrinck AM, Delzenne NM, Knauf C, Cani PD (2014) Gut microbiota controls adipose tissue expansion, gut barrier and glucose metabolism: novel insights into molecular targets and interventions using prebiotics. Benef Microbes 5(1):3–17

Gouyer V, Dubuquoy L, Robbe-Masselot C, Neut C, Singer E, Plet S, Geboes K, Desreumaux P, Gottrand F, Desseyn J-L (2015) Delivery of a mucin domain enriched in cysteine residues strengthens the intestinal mucous barrier. Sci Rep 5:9577

Grenham S, Clarke G, Cryan JF, Dinan TG (2011) Brain–gut–microbe communication in health and disease. Front Physiol 2:94

Guerra A, Etienne-Mesmin L, Livrelli V, Denis S, Blanquet-Diot S, Alric M (2012) Relevance and challenges in modeling human gastric and small intestinal digestion. Trends Biotechnol 30(11): 591–600

Hamady M, Knight R (2009) Microbial community profiling for human microbiome projects: Tools, techniques, and challenges. Genome Res 19(7):1141–1152

Hayashi H, Takahashi R, Nishi T, Sakamoto M, Benno Y (2005) Molecular analysis of jejunal, ileal, caecal and recto-sigmoidal human colonic microbiota using 16S rRNA gene libraries and terminal restriction fragment length polymorphism. J Med Microbiol 54(11):1093–1101

Heijtz RD, Wang S, Anuar F, Qian Y, Björkholm B, Samuelsson A, Hibberd ML, Forssberg H, Pettersson S (2011) Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci 108(7):3047–3052

Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JI (2001) Molecular analysis of commensal host-microbial relationships in the intestine. Science 291(5505):881–884

Hueston CM, Deak T (2014) The inflamed axis: the interaction between stress, hormones, and the expression of inflammatory-related genes within key structures comprising the hypothalamic–pituitary–adrenal axis. Physiol Behav 124:77–91

Iwasaki A, Medzhitov R (2010) Regulation of adaptive immunity by the innate immune system. Science 327(5963):291–295

Kang D-W, Park JG, Ilhan ZE, Wallstrom G, LaBaer J, Adams JB, Krajmalnik-Brown R (2013) Reduced incidence of Prevotella and other fermenters in intestinal microflora of autistic children. PLoS One 8(7):e68322

Kedinger M, Simon-Assmann P, Alexandre E, Haffen K (1987) Importance of a fibroblastic support for in vitro differentiation of intestinal endodermal cells and for their response to glucocorticoids. Cell Differ 20(2-3):171–182

Koch H, Schmid-Hempel P (2011) Bacterial communities in central European bumblebees: low diversity and high specificity. Microb Ecol 62(1):121–133

Kopp ZA, Jain U, Van Limbergen J, Stadnyk AW (2015) Do antimicrobial peptides and complement collaborate in the intestinal mucosa? Front Immunol 6:17

Kumamoto CA (2011) Inflammation and gastrointestinal Candida colonization. Curr Opin Microbiol 14(4):386–391

Kuraishi T, Binggeli O, Opota O, Buchon N, Lemaitre B (2011) Genetic evidence for a protective role of the peritrophic matrix against intestinal bacterial infection in *Drosophila melanogaster*. Proc Natl Acad Sci 108(38):15966–15971

Kuraishi N, Matsui M, Hamidy A, Belabut DM, Ahmad N, Panha S, Sudin A, Yong HS, Jiang JP, Ota H (2013a) Phylogenetic and taxonomic relationships of the Polypedates leucomystax complex (Amphibia). Zool Scr 42(1):54–70

Kuraishi T, Hori A, Kurata S (2013b) Host-microbe interactions in the gut of *Drosophila melanogaster*. Front Physiol 4:375

Lee K-A, Kim S-H, Kim E-K, Ha E-M, You H, Kim B, Kim M-J, Kwon Y, Ryu J-H, Lee W-J (2013) Bacterial-derived uracil as a modulator of mucosal immunity and gut-microbe homeostasis in *Drosophila*. Cell 153(4):797–811

Lemaitre B, Hoffmann J (2007) The host defense of *Drosophila melanogaster*. Annu Rev Immunol 25:697–743

Lemaitre B, Miguel-Aliaga I (2013) The digestive tract of *Drosophila melanogaster*. Annu Rev Genet 47:377–404

Leone V, Gibbons SM, Martinez K, Hutchison AL, Huang EY, Cham CM, Pierre JF, Heneghan AF, Nadimpalli A, Hubert N (2015) Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock function and metabolism. Cell Host Microbe 17(5):681–689

Liehl P, Blight M, Vodovar N, Boccard F, Lemaitre B (2006) Prevalence of local immune response against oral infection in a *Drosophila*/Pseudomonas infection model. PLoS Pathog 2(6):e56–e56

Liévin-Le Moal V, Servin AL (2014) Anti-infective activities of lactobacillus strains in the human intestinal microbiota: from probiotics to gastrointestinal anti-infectious biotherapeutic agents. Clin Microbiol Rev 27(2):167–199

Liévin-Le Moal V, Amsellem R, Servin AL (2011) Impairment of swimming motility by antidiarrheic Lactobacillus acidophilus strain LB retards internalization of Salmonella enterica serovar Typhimurium within human enterocyte-like cells. Antimicrob Agents Chemother 55(10):4810–4820

Lombardo MP (2008) Access to mutualistic endosymbiotic microbes: an underappreciated benefit of group living. Behav Ecol Sociobiol 62(4):479–497

MacDonald TT, Monteleone G (2005) Immunity, inflammation, and allergy in the gut. Science 307(5717):1920–1925

Marteau P, Lepage P, Mangin I, Suau A, Dore J, Pochart P, Seksik P (2004) Gut flora and inflammatory bowel disease. Aliment Pharmacol Ther 20(s4):18–23

Mayer EA (2011) Gut feelings: the emerging biology of gut–brain communication. Nat Rev Neurosci 12(8):453–466

McClements DJ, Li Y (2010) Review of in vitro digestion models for rapid screening of emulsion-based systems. Food Funct 1(1):32–59

McFall-Ngai M (2007) Adaptive immunity: care for the community. Nature 445(7124):153–153

McFall-Ngai M, Hadfield MG, Bosch TC, Carey HV, Domazet-Lošo T, Douglas AE, Dubilier N, Eberl G, Fukami T, Gilbert SF (2013) Animals in a bacterial world, a new imperative for the life sciences. Proc Natl Acad Sci 110(9):3229–3236

Messaoudi M, Lalonde R, Violle N, Javelot H, Desor D, Nejdi A, Bisson J-F, Rougeot C, Pichelin M, Cazaubiel M (2011) Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. Br J Nutr 105(05):755–764

Montiel-Castro AJ, González-Cervantes RM, Bravo-Ruiseco G, Pacheco-López G (2013) The microbiota-gut-brain axis: neurobehavioral correlates, health and sociality. Front Integr Neurosci 7:70

Moran NA, McCutcheon JP, Nakabachi A (2008) Genomics and evolution of heritable bacterial symbionts. Annu Rev Genet 42:165–190

Nehme NT, Liégeois S, Kele B, Giammarinaro P, Pradel E, Hoffmann JA, Ewbank JJ, Ferrandon D (2007) A model of bacterial intestinal infections in *Drosophila melanogaster*. PLoS Pathog 3(11):e173

Neish AS (2009) Microbes in gastrointestinal health and disease. Gastroenterology 136(1):65–80

Neyen C, Poidevin M, Roussel A, Lemaitre B (2012) Tissue-and ligand-specific sensing of gram-negative infection in *Drosophila* by PGRP-LC isoforms and PGRP-LE. J Immunol 189(4): 1886–1897

Nichols CD, Becnel J, Pandey UB (2012) Methods to assay *Drosophila* behavior. J Vis Exp 61: 3795

Nicolas V, Liévin-Le Moal V (2015) Antisecretory factor peptide af-16 inhibits the secreted autotransporter toxin-stimulated transcellular and paracellular passages of fluid in cultured human enterocyte-like cells. Infect Immun 83(3):907–922

Overend G, Luo Y, Henderson L, Douglas AE, Davies SA, Dow JA (2016) Molecular mechanism and functional significance of acid generation in the *Drosophila* midgut. Sci Rep 6:27242

Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO (2007) Development of the human infant intestinal microbiota. PLoS Biol 5(7):e177

Panayidou S, Apidianakis Y (2013) Regenerative inflammation: lessons from *Drosophila* intestinal epithelium in health and disease. Pathogens 2(2):209–231

Phumkhachorn P, Rattanachaikunsopon P, Khunsook S (2007) Use of the gfp gene in monitoring bacteriocin-producing Lactobacillus plantarum N014, a potential starter culture in nham fermentation. J Food Prot 70(2):419–424

Pitsouli C, Apidianakis Y, Perrimon N (2009) Homeostasis in infected epithelia: stem cells take the lead. Cell Host Microbe 6(4):301–307

Priyadarsini S, Sahoo M, Sahu S, Jayabalan R, Mishra M (2019) An infection of Enterobacter ludwigii affects development and causes age-dependent neurodegeneration in Drosophila melanogaster. Invert Neurosci 19(4):13

Priyadarsini S, Mukherjee S, Samikshya S, Bhanja A, Paikra SK, Nayak N, Mishra M (2020) Dietary infection of Enterobacter ludwigii causes fat accumulation and resulted in the diabetes-like condition in Drosophila melanogaster. Microb Pathog 149:104276

Rahman MM, McFadden G (2011) Modulation of NF-κB signalling by microbial pathogens. Nat Rev Microbiol 9(4):291–306

Ranjan R, Abhinay A, Mishra M (2018a) Can oral microbial infections be a risk factor for neurodegeneration? A review of the literature. Neurol India 66(2):344

Ranjan R, Dhar G, Sahu S, Nayak N, Mishra M (2018b) Periodontal disease and neurodegeneration: the possible pathway and contribution from periodontal infections. J Clin Diagn Res 12(1):DE01–DE05

Rubin DC (2007) Intestinal morphogenesis. Curr Opin Gastroenterol 23(2):111–114

Ryu J-H, Ha E-M, Lee W-J (2010) Innate immunity and gut–microbe mutualism in *Drosophila*. Dev Comp Immunol 34(4):369–376

Sabat D, Johnson E, Abhinay A, Jayabalan R, Mishra M (2015) A protocol to generate germ free *Drosophila* for microbial interaction studies. Adv Tech Biol Med 1:2379–1764

Salminen S, Nybom S, Meriluoto J, Collado MC, Vesterlund S, El-Nezami H (2010) Interaction of probiotics and pathogens—benefits to human health? Curr Opin Biotechnol 21:157–167

Shanbhag S, Tripathi S (2009) Epithelial ultrastructure and cellular mechanisms of acid and base transport in the *Drosophila* midgut. J Exp Biol 212(11):1731–1744

Shibata T, Sekihara S, Fujikawa T, Miyaji R, Maki K, Ishihara T, Koshiba T, Kawabata S-i (2013) Transglutaminase-catalyzed protein-protein cross-linking suppresses the activity of the NF-κB–like transcription factor relish. Sci Signal 6(285):ra61

Shin SC, Kim S-H, You H, Kim B, Kim AC, Lee K-A, Yoon J-H, Ryu J-H, Lee W-J (2011) *Drosophila* microbiome modulates host developmental and metabolic homeostasis via insulin signaling. Science 334(6056):670–674

Simhadri RK, Fast EM, Guo R, Schultz MJ, Vaisman N, Ortiz L, Bybee J, Slatko BE, Frydman HM (2017) The gut commensal microbiome of Drosophila melanogaster is modified by the endosymbiont wolbachia. mSphere 2(5):e00287–e00217

Spooner R, Yilmaz Ö (2011) The role of reactive-oxygen-species in microbial persistence and inflammation. Int J Mol Sci 12(1):334–352

Stilling RM, Dinan TG, Cryan JF (2014) Microbial genes, brain & behaviour–epigenetic regulation of the gut–brain axis. Genes Brain Behav 13(1):69–86

Takeuchi O, Akira S (2010) Pattern recognition receptors and inflammation. Cell 140(6):805–820

Thirabunyanon M (2011) Biotherapy for and protection against gastrointestinal pathogenic infections via action of probiotic bacteria. Maejo Int J Sci Technol 5(1):108–128

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. Appl Biochem Biotechnol 169(2):511–525

Thompson D, Richelson E, Malagelada J (1983) Perturbation of upper gastrointestinal function by cold stress. Gut 24(4):277–283

Thomson RM, Armstrong JG, Looke DF (2007) Gastroesophageal reflux disease, acid suppression, and Mycobacterium avium complex pulmonary disease. Chest J 131(4):1166–1172

Trinder M, Daisley BA, Dube JS, Reid G (2017) Drosophila melanogaster as a high-throughput model for host–microbiota interactions. Front Microbiol 8:751

Troyer K (1984) Microbes, herbivory and the evolution of social behavior. J Theor Biol 106(2): 157–169

Tsabouri S, Priftis K, Chaliasos N, Siamopoulou A (2014) Modulation of gut microbiota downregulates the development of food allergy in infancy. Allergol Immunopathol 42(1):69–77

Varyukhina S, Freitas M, Bardin S, Robillard E, Tavan E, Sapin C, Grill J-P, Trugnan G (2012) Glycan-modifying bacteria-derived soluble factors from Bacteroides thetaiotaomicron and Lactobacillus casei inhibit rotavirus infection in human intestinal cells. Microbes infect 14: 273–278

Whitcomb DC, Lowe ME (2007) Human pancreatic digestive enzymes. Dig Dis Sci 52:1–17

Wong AC, Vanhove AS, Watnick PI (2016) The interplay between intestinal bacteria and host metabolism in health and disease: lessons from *Drosophila melanogaster*. Dis Model Mech 9(3):271–281

Yerkes RM, Dodson JD (1908) The relation of strength of stimulus to rapidity of habit-formation. J Comp Neurol 18(5):459–482

Zasloff M (2002) Antimicrobial peptides of multicellular organisms. Nature 415(6870):389–395

15 Prospectives of Prebiotics, Probiotics, and Synbiotics for Sustainable Development of Aquaculture

P. Kaushik, R. Khandelwal, N. Jain, S. Keelka, and P. Jain

Abstract

Aquaculture is a rapidly mounting industry which provides high-quality protein worldwide as 70% of the world population depends on aquaculture food products. The major concerns causing imbalance in the industry include infections caused by experimental manifestation coupled with opportunistic pathogens, weak immune systems, and stress conditions leading to drastic economic instability.

Although antibiotics has been introduced for resolving the subject and setting efforts toward sustainable practices, the fear of antibiotic-resistant microbes, underdeveloped immune system, and persistence of drug residues in the flesh has been widely witnessed. This has paved a way toward an alternative approach by administration of prebiotics, probiotics, and synbiotics as dietary supplements to the cultured species.

Prebiotics are selective food ingredients obtained by fermentation process using plants or sugar conversed food materials. The administration of prebiotics in aquaculture is useful as these act as immune stimulants and increase growth and activity of microflora present in the intestine which are essential to maintain good health conditions of the host. The accomplishment in health improvement and reducing pathogen susceptibility strategy marks prebiotics as promising candidates toward eco-friendly feed-supportive additives in the industry of aquaculture.

The probiotics are live microorganisms which restore the balance between good and bad bacteria in the gut by secreting bactericidal proteins and decreasing the pH which renders the infectious bacteria unable to survive in that environment; these also block the attachment and invasion of epithelium by pathogens.

P. Kaushik (✉) · R. Khandelwal · N. Jain · S. Keelka · P. Jain
Centre for Advanced Studies, Department of Zoology, University of Rajasthan, India, Jaipur

K. K. Behera et al. (eds.), *Prebiotics, Probiotics and Nutraceuticals*,
https://doi.org/10.1007/978-981-16-8990-1_15

Synbiotics alludes to synergism, which is a combination of positive microbial supplements prebiotic and probiotic. Synbiotics is characterized by some colonic foods with nutritional properties to maintain intestinal homeostasis and healthy body of aqueous organisms; hence it is called health-enhancing food ingredient. Synbiotics can be utilized in aquaculture to measure numerous contexts like skin mucous immune response (lysozyme activity, total immune globulin, and protease), stress resistance, temperature, salinity, intestinal microbiota maintenance, and growth indices at the end of feeding trials of organisms.

This stratagem not only ameliorates growth and encourages immunity but also ensures intestinal health in opposition to pathogens.

Keywords

Integrated multitrophic aquaculture · Growth promotion · Commercial products · Mode of action · Immunomodulation

15.1 Introduction

Aquaculture is the practice of rearing, harvesting, and breeding of aquatic species like fish, crustaceans, mollusks, and plants in controlled aquatic milieus such as fresh water, brackish water, or salt water (Development of Marine and Inland Aquaculture 2020). This is considered as aquatic counterpart of agriculture in which planned farming of aquatic organisms can be performed in aquatic system to support the food requirement of growing population worldwide. The human civilization has greatly harnessed the aquatic system as food sources since ages, but much of the development in aquatic farming has been witnessed post-World War II. This is one of the industries which can not only provide protein-rich food specially for the local population residing along the coast or shores but also provide employment and trade along with sustainable use of sea resources. The yearlong regular supply of seafood can help fight the menace of malnutrition among poor as fish provides a good source of vitamins A, B, and D, calcium, iron, and iodine along with essential amino acids. Moreover, there exists a wide range of non-food purposes of aquaculture products such as pharmaceuticals, cosmeceuticals, nutraceuticals, ornamentals, environmental conservation, academics, and even the production of biofuels.

The aquaculture involves various culturable practices depending on the aquatic conditions, aim of farming, and the available tools of operation chiefly into mariculture, fish farming, algaculture, and integrated multitrophic aquaculture (Fig. 15.1). *Mariculture* majorly involves seawater either within an ocean partitioned portion or in ponds. The species which are bred vary from seaweed, mollusks, and even seafood alternatives. The *fish farming* mainly involves fish breeding in selective manner either in seawater or fresh water. It is the most common type of aquaculture which is exceedingly exploited as this provides a very good and cheap source of protein and does not require much space and care. In *algaculture* cultivation of algae is done. There are multiple uses of various types of algae; thus for economic

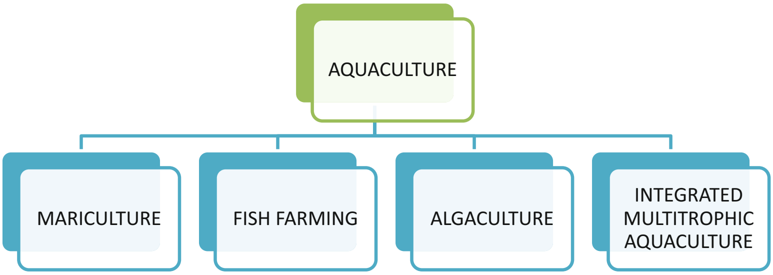


Fig. 15.1 Different types of aquaculture practices

viability, it is grown and harvested in significant quantities. The *integrated multitrophic aquaculture* is an advanced aquaculture system in which multiple trophic levels are incorporated into the network to meet varying nutrient requirements for them. This provides an efficient strategy as it mimics the natural biological environment which leads to utilization of squander of larger organisms as feeding resource for smaller organisms.

Aquaculture of any of the above-mentioned types basically involves a human interference with breeding and harvesting of either indigenous, exotic, or even endangered/threatened species. In natural, modified, or artificially designed system, there is a reasonable control of physical and chemical parameters of water. The intensity of human intervention in the natural or artificial reading conditions of aquaculture becomes the basis of its categorization as semi-intensive, moderately intensive, and highly intensive. The higher the intensity of intervention, the greater the cost of culturing, maintaining, and harvesting. The organisms to be cultured are either added as seed/egg in the system or in larval stages and provided with intensive food supply along with protection from predators and competition. For making the entire provision cost-effective, the maximum utilization of resources is ensured by increasing the rate of growth and number of individuals per unit area.

The magnitude of this industry can be estimated in terms of total production and economic values. According to the report of FAO (2020) on the State of World Fisheries and Aquaculture, the global fish production including crustaceans, mollusks, and other aquatic animals has been estimated to have reached about 179 million tons in 2018 accounting to total first sale value of about USD 401 billion. The aquaculture comprises about half (46%, 82 million tons) of the total production (179 million tons) with estimated value of USD 250 billion. FAO (2020) provides assumptions about 32% increase of aquaculture production (26 million tons) over the 2018 production reaching to 109 million tons in 2030 with an average growth of 2.3%.

15.1.1 Negative Impact

The negative impact of extensive commercial aquaculture on the ecosystem and biodiversity also needs to be taken into account, like destruction of coastal habitat, compromise in water quality, and lowering conspecifics fitness due to genetic pollution (Gentry et al. 2020). The major consequence is the infections caused by several pathogenic microorganisms and also via the different experiments executed on the cultured species that cause traumatic circumstances in the body system (Bentzon-Tilia et al. 2016; Amenyogbe et al. 2020). Predominantly pathogenic *Vibrio* spp. which are naturally allied with zooplanktons effortlessly proliferate in phytoplankton that are the live feed for the culture, making them as opportunistic vectors. These consequences pave the way toward the need of practices which can be a sustainable approach (Dittmann et al. 2020) to maximize the availability of goods and beneficial services and minimize negative repercussions (Gentry et al. 2020).

15.1.2 Challenges of Sustainable Aquaculture

In order to provide sustainable nutritional requirement of the growing population with a projected rise of world population to about 9.6 billion people in 2050, agriculture and aquaculture are desired to increase production levels (Small et al. 2016). Although significant advances have been scrutinized to ensure high productivity and quality along with considering fish welfare, monitoring interactions among nutrition, genotype, and environment and controlling major water parameters cumulatively affect the cultivations and thus are key concerns for sustainable development.

15.1.2.1 Feed

There is a broad inconsistency in feeding modalities, diet composition, and the administration time which elicit significant influence on fish behavior and growth (Toni et al. 2017). One of the most imperative enhancing knowledge in aquaculture is the espousal of cooking-extrusion technology for producing pelleted feeds. This technology has more advantages than the earlier used feeding modalities due to its durable nature, improved water stability, increased starch gelatinization, and high intact life to make it possible for slow feeders, e.g., catfish and shrimp, for an effective and increased consumption. Another technology that has achieved a standard position in the industry is the development of micro diets for the fishes that endure larval stages. Before the adoption of this technology, larvae were fed live prey like *Artemia nauplii* and rotifers as they were not enough large for the conventional feed which used to result in high mortality (Small et al. 2016). Moreover, overfeeding leads to increased production of fecal matter and waste that leads to deoxygenation of water and production of reduced compounds like sulfides and ammonium, whereas underfeeding causes inter-fish competition and attack events which can be a cause of severe injuries.

15.1.2.2 Organic Compounds

Another factor is organic compound concentration as its increased levels could be detrimental for the aquatic animals as well as organisms which feed on them such as humans. Ammonia, a product of nitrogenous compound metabolism, is highly soluble and toxic and can cause relentless, histopathological changes and other stressful effects leading to altered oxygen delivery, modified amino acid metabolism, increased cortisol levels, and compromised food intake (Toni et al. 2017). Other organic compounds like polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), brominated flame retardants (BFRs), and organochlorinated pesticides (OCPs) are the residues of antibiotics and veterinary drugs that have the potential to enter the aquaculture system through feeding and gets accumulated in the food chain, out of which aldrin (examples of OCPs) and DDT (dichlorodiphenyltrichloroethane) have the highest power to accrue in fatty tissues of the organism (Su et al. 2020).

15.1.2.3 Infectious Diseases

The management of infectious ailments for sustainability of large-scale aquaculture, regarding economic welfare and global food security, is a critical challenge. Depending on the severity of contamination, some pathogens like *Vibrio vulnificus*, *Streptococcus iniae*, and *Mycobacterium marinum* infect both humans and fishes, whereas some pathogens including *Aeromonas salmonicida*, *Vibrio harveyi*, and *Vibrio anguillarum* cause infection only in fishes. *Escherichia coli*, *Vibrio cholerae*, and *Salmonella* spp. are some examples which cause infection only in human via consumption of contaminated fish products, but do not influence fish survival. One of the most serious pathogens in shellfish and fish aquaculture is *Vibrio* spp., a genus of gram-negative bacteria. Other death-causing pathogens include *Vibrio harveyi*, *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus*, *V. anguillarum*, and *V. salmonicida* (Su et al. 2020). In some cases, surviving exposure to an infection makes the organism resistant by acquiring long-term immunity against the pathogen, like in the case of a ciliated protozoan parasite *Ichthyophthirius multifiliis* (Alves and Ta 2020). Potential risk could also be posed by insufficient knowledge about the temporal variation and diversity of pathogenic communities, like bacteria especially *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* that reside in the shrimp's intestinal tract and significantly alter from juvenile to adult. For the sake of food safety and human health, proper knowledge for antibiotic treatment should be taken into consideration (Su et al. 2017).

The dramatic increase in the ailments and eruptions could be calamitous for the commerce. The Chilean infectious salmon anemia (ISA) epidemic of 2007 decimated the business and left US $1.8 billion in debt. Low rural income groups were faultily affected with the loss of more than 13,000 employers (Bayliss et al. 2017). The resurgence of pathogens may arise from the propagation of novel strains or through the international dissemination and spread of putative pathogens. New strains that cause an emerging research can foreseeably appear via host switching due to long-term mixed farming, or conversely new pathogen strains from the surroundings of commensal organisms might develop. This can occur as a consequence of mutation or recombination of virulence gene leading toward horizontal acquisition between populations of previously isolated pathogen. The effects of vulnerability to pathogens in aquaculture settings are frequently perilously reliant on the temperature and dissolved oxygen levels in the water. The temperature of water influences pathogen replication rate as well as immune responses of the poikilothermic fishes, while some of the bacterial pathogens are virulent at cold temperatures (10 °C) like *Flavobacterium psychrophilum* and some at more than 15 °C such as *Lactococcus garvieae* (Bayliss et al. 2017).

Another pathogen that is currently affecting aquaculture industries is COVID-19, a virus that does not directly influence the organism's anatomy but does impinge on the commercial trade. As per the outcome of the global pandemic, it is hard for the business to survive in the long term and cope with the major market loss (Senten et al. 2020).

15.1.2.4 Microbial Antibiotic Resistance (MAR)

One of the global, critical threats inscribed in aquaculture industry is the exploitation of antibiotics and antimicrobial resistance (AMR) (Watts et al. 2017; Preena et al. 2020b; Thornber et al. 2020). The prevalence of bacterial infections in cultured organisms encourages the continuous use of antibiotics that results in the emergence of antibiotic-resistant bacteria and their potential spread in the entire aquatic ecosystem by the horizontal gene transfer (Watts et al. 2017; Preena et al. 2020b). The intensification of culture systems for greater yields elevates nutrient pollution, stocking density dwindles the water quality, and any pathogen outbreaks increases reliance on antibiotics and supplements (Watts et al. 2017). Indiscriminate antimicrobial use contributes to discriminatory stress amplification which eventually results in breakout. This reflects the risk of transferring antibiotic-resistant gene to the terrestrial world, which should be regarded as critical as this influences the public health issues (Preena et al. 2020b). The prolonged use of antimicrobials leads to antimicrobial-resistant bacteria and antibiotic selection pressure. The higher pathogens, MAR index, and MIC (minimum inhibitory concentration) of antibiotics indicate the rigorous use of antibiotics. The ability of the pathogen to depict tolerance even to the latest generation of antibiotics is a reminder of the need for careful antibiotic usage and control programs with constant surveillance (Preena et al. 2020a). An approximate of 90% of bacteria found in seawater is immune to one or more types of antibiotics, and it is also evident that around 20% bacteria are resistant to about five types of antibiotics. Bacteria acquired with MAR genes remain in the atmosphere for long period of time, even after the pressure for selection ceases. Done and Halden in 2015 reported reduced but substantial levels of macrolide, tetracycline, and sulfonamide named antibiotics in field samples of tilapia, trout, and salmon from around 11 countries including Thailand, Canada, Mexico, and the United States (Watts et al. 2017). It is also evident that elevated death rates of infected fishes occur at warmer temperature especially in the countries with vulnerable climate change, antibiotic misuse, or poor sanitation, as in Africa and Asia (Reverter et al. 2020). The development and prevalence of AMR in ecosystem is a public health issue, with an approximately 700,000 deaths currently reported annually and projected 12 million deaths per annum in 2050. In addition, by 2050, reduced economic production could cost AMR $100 trillion. Consequently it is important to comprehend environmental hotspots for AMR genetic exchange (Watts et al. 2017). To reach our aim of speeding industry production, it is vital to recognize sinks and sources of antimicrobial resistance and scrutinize with frequent assessment of transfer of AMR between the communities of microbes, farmed products, and the environmental components (Watts et al. 2017; Preena et al. 2020a). Advanced microbiological, omic-based, and molecular biology contrivances can disentangle the challenge to a certain degree. Moreover, mobile genetic elements like plasmids, integrons, gene cassettes, and transposons of the AMR determinants need to be examined and monitored for apposite implementation of management procedures with satisfactory substitute strategies in the industry. Also the antibiotic susceptibility needs to be tested with various conjugation and biochemical assays to verify the degree of AMR spread (Preena et al. 2020b).

Thus, other sustainable solutions to increase system resilience and lessen antibiotic use with animal welfare consideration are the need of the hour. The antimicrobial drugs, pesticides, and agents used for disinfection are used in aquaculture for the prevention of diseases, but this adds to the progression of resistant strains of bacteria.

15.1.3 Alternative Strategies for Sustainable Aquaculture

Alternative, environment-friendly, biological approaches can provide an edge over the conventional treatment approaches which can support the culturable organisms to fight against the pathogens. The use of natural beneficial microbes (probiotics) or non-digestible dietary substances (prebiotics) or a combination of both (synbiotics) has been tested extensively in many animal and human diseases. These have also been applied on various aquatic culturable forms to study the effectiveness in prevention, severity, or recovery from disease condition. Using either of the strategies to provide protection against pathogen as well as proper growth is considered as a sustainable strategy as these are cost-effective, renewable, and environmentally friendly.

The details of all these sustainable strategies along with their beneficial effects and application in aquaculture are provided in the following text.

15.2 Prebiotics

The prebiotics concept was presented by Gibson and Roberfroid (1995) in their scientific publication called "Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics." Prebiotics was described as a food which is not digested and that positively influences the health of specific bacteria in the colon by stimulating their growth that in turn improves the host health. The scientific community has been discussing the prebiotic substances from the last few decades that target human health. The meeting of ISAPP (International Scientific Association of Probiotics and Prebiotics) presented the prebiotic substances as a particularly fermented substrates that result in peculiar changes in microbiota of gut and confers health advantages for the organism (Gibson et al. 2010; ISAPP 2006). The prebiotic definition may additionally evolve as science advances, and it is required for the scientific community to clarify new findings and modify the prebiotic concept. The purpose of prebiotics is to enhance specific beneficial indigenous microbiota population.

There are few criteria (Gibson et al. 2004) to select substances as a prebiotic compound which include:

1. The substance is selected as a prebiotic if it is resistant to the acidic pH of abdomen and it does not get hydrolyzed or absorbed in the GI tract.
2. It should be fermented by the microorganisms of the GI tract.
3. It should stimulate the activity of growth in intestinal bacteria that in turn improves the health of host organism.

Types of prebiotics: There are various categories of prebiotics, but most of these comprise a group of carbohydrates consisting mostly of oligosaccharides. Prebiotics can be either naturally occurring substances such as banana, asparagus, beans, mushrooms, onion, garlic, savory, and cereals or can also be produced by the enzymatic digestion of various polysaccharides; some other specific complex substances are available in the market in the form of oligosaccharides (Davani-Davari et al. 2019).

The most common categories of prebiotics are mentioned below.

15.2.1 Fructans

Fructans is the category which includes inulin and fructo-oligosaccharide (FOS) (Davani-Davari et al. 2019). Inulin is longer in size than FOS; inulin is naturally found in chicory and artichoke. FOS exists in about 36,000 plants; garlic and onions are the common foods which contain FOS. However, the required amount of FOS to act as prebiotic in these natural sources in not enough. Therefore, FOS is synthesized by different production methods (Sangeetha et al. 2005). Fructosyltransferase (FTase) is a crucial enzyme in the production of FOS. Several microbial species have been reported which contains FTase such as *Aspergillus* sp. and *Saccharomyces cerevisiae* (Yun 1996; Sangeetha et al. 2005). The expeditious methods for FTase-producing microbes' screening are associated to techniques on recombinant DNA which permit the selection of new highly efficient strains.

15.2.2 Galacto-oligosaccharides (GOS)

These are fabricated from extension of lactose which are classified into two subgroups, i.e., (A) GOS with extra quantity of galactose at C_3, C_4 or $C_6.$ and

(B) the GOS produced from lactose via enzymatic trans-glycosylation, and this type of GOS is also termed as trans-galacto-oligosaccharides (TOS) (Gibson et al. 2010).

The enzymes involved in the production of GOS are galactosidase and galactosyl-transferase. Formation of GOS via galactosidase is much inexpensive as compared to galactosyl-transferase. β-Galactosidase activity has been reported from different sources such as *Aspergillus oryzae*, *Lactobacilli*, and *S. cerevisiae*. Recombinant β-galactosidase is much more effective than the natural source. Recombinant β-galactosidase has higher production yield and easy purification and also contains improved enzymatic stability. Baker's yeast has been used as a recombinant β-galactosidase-producing microorganism (Demain and Vaishnav 2009). Some GOS derived from lactulose are also considered as prebiotics.

15.2.2.1 Glucose-Derived Oligosaccharides

Polydextrose: Polydextrose is a glucose derivative. It contains glycosidic linkage. It selectively stimulates the growth of intestinal bacteria (do Carmo et al. 2016).

Resistant starch: Resistant starch can enhance health by generating an excessive amount of butyrate, so it has been recommended to be categorized as a prebiotic (Zaman and Sarbini 2016).

Pectic oligosaccharide: This has also been considered as a prebiotic compound due to its anti-inflammatory properties, and it also stimulates the apoptosis of colon cancer (Hotchkiss et al. 2003).

15.2.2.2 Others

Cocoa-derived flavanols are not carbohydrates but can be classified as prebiotics as few experiments have revealed that cocoa-derived flavanols stimulate lactic acid bacteria in animals (Tzounis et al. 2011).

15.2.2.3 Commercial Prebiotics

Grobiotic®-A: It is a commercial available prebiotic which is a combination of moderately autolyzed brewer's yeast, dairy additives, and dried products of fermentation (Li and Gatlin 2005).

MacroGard®: It is a reservoir of extremely purified, liable, and conserved β 1,3/1,6 glucans comprised of exclusively selected strain of yeast *Saccharomyces cerevisiae*. It acts as an immune-modulating compound used in aquaculture and animal husbandry (Sealey et al. 2008; Meena et al. 2013).

Alginate: Alginate oligosaccharide acts as a growth stimulator and immunostimulant (Gupta et al. 2019).

Levabon® Aquagrow E (*Biomin®, Austria*): It is a commercial prebiotic which acts as an immune modulator in the modern aquaculture. It contains appropriate amount of β-glucans, mannan oligosaccharides, and nucleotides with increased bioavailability which work in synergy to enhance the immune system (Passos 2017).

15.2.3 Application of Prebiotics on Aquatic Species

15.2.3.1 Effect on Digestive Performance

Prebiotics particularly stimulate the metabolism and promote one or more constrained ranges of useful and health-promoting bacteria in the gut. Dietary mannan oligosaccharide (MOS) can influence intestinal morphology in the anterior and posterior regions with increase in the absorptive surface area by promoting longer mucosal folding in the rainbow trout (*Oncorhynchus mykiss*) (Dimitroglou et al. 2009). Positive impact of MOS has been reported on community of lactic acid-producing bacteria in common carp (Momeni-Moghaddam et al. 2015). Some bacteria have the ability of recognizing the binding sites on the prebiotics as a substitute of intestinal mucosa; therefore, besides a decrease in infection frequency, there is also a notable enhancement within the absorption capability of nutrients which are available (Radecki and Yokoyama 1991). Although, there is some disagreement in the scientist community regarding beneficial effect on intestine as Santin et al. (2001) observed no significant difference in ileal villi length at 28 and 42 day in broiler chicken using prebiotics (Ganguly et al. 2013). In another research, FOS has proven to increase feed performance in blunt snout bream *Megalobrama amblycephala*. Savory enhances lactic acid bacteria in the intestine of common carp that in turn increases mineral absorption (Mousavi et al. 2016). The low level of macroalga-derived alginate oligosaccharide can also act as a prebiotic for the distal intestinal microbiota in Atlantic salmon (Gupta et al. 2019).

15.2.3.2 Growth Performance

Prebiotics can be considered as a growth factor to particular commensal bacterial organisms. Research has proven that the nutritional supplementation with a commercially available prebiotic has extensively increased disease resistance and growth of hybrid striped bass beyond that carried out with brewer's yeast (Li and Gatlin 2005). MOS dietary and water supplementation have improved the growth and weight gain in the Nile tilapia (*Oreochromis niloticus*) (Wakeel et al. 2019). Supplementation with prebiotics had a strong effect on growth performance and survival rate of *Channa striata* (Munir et al. 2016). Levabon® Aquagrow E (Biomin®, Austria) has been successfully investigated for improving the growth performance in the fish to reach the marketable size in shorter time duration as compared to the untreated ones.

15.2.3.3 Immunostimulation

Prebiotics is an important factor in controlling the disease. Numerous polysaccharides from different sources have the capability to trigger the immune system against infections (Meena et al. 2013). β-Glucans play a crucial role in the stimulation of immune system in opposition to the bacterial and viral infections in the fin fishes and crustaceans (Soltanian Stuyven et al. 2009; Meena et al. 2013). MacroGard® which is produced from the baker's yeast also acts as alternative to antibiotics in aquaculture (Soltanian Stuyven et al. 2009; Meena et al. 2013). Alginate which is also a prebiotic supplement acts as an immune-stimulator for the fishes in the development stage when adaptive immune system is less developed. High mannuronic acid containing alginate has also been used as an immunostimulant for improvement of innate immune resistance in the fish larvae and fry (Ringø et al. 2014). One percent dietary inulin improve the health of fish by enhancing immune response (Mousavi et al. 2016). Levabon® Aquagrow E (Biomin®, Austria) works as an immune-stimulator in juvenile European sea bass (*Dicentrarchus labrax*) (Passos 2017).

15.2.3.4 Effect of Prebiotics on the Biochemical Parameters

Prebiotic substance intake also affects cholesterol, insulin, and other biochemical parameters of aquatic animals. Higher serum protein level was recorded in juvenile beluga fed with inulin (Akrami et al. 2009), and the same effects were obtained when onion and garlic were given to catfish (Al-Salahy 2002). Bioactive components of onion such as methiin and S-allyl cysteine sulfoxide (SACS) exert their anti-diabetic action by stimulating the insulin production and increasing the absorption of dietary glucose (Srinivasan 2005). Onions have a capability to reduce triglycerides in blood (Effendy et al. 1997). Allicin and its derivatives present in onion and garlic are responsible for the hypolipidemic effects in human as well as in experimental animals (Liu and Yeh 2002). Calcium and phosphorus levels increased in common carp when it was supplemented with inulin and savory diet (Mousavi et al. 2016). The fermentation of prebiotics increase the amount of short-chain fatty acid such as acetate, butyrate, and propionate that induce low pH in gut and consequently increase the absorption; decreased pH provides the optimal condition to the lactic acid bacteria in the gut of fish (Scholz-Ahrens et al. 2001).

15.2.4 Mechanism of Action

Fermentation is the main mechanism of action by which prebiotics are being utilized by the beneficial bacteria of colon (Slavin 2013). The intestinal flora retrieve energy via fermentation of carbohydrates which escape digestion in the upper GI tract. The primary substrates for these microorganisms are dietary carbohydrates that are not digested in the upper gut. Dietary components that trigger fermentation will increase the bacterial mass and short-chain fatty acid production. Butyrate is used by colonic epithelial cells as an energy source and is also an important nutrient determining the metabolic activity (Lupton 2004). Fermentation (production of acetic, propionic, butyric acid) and short-chain fatty acid production obstruct the growth of harmful pathogenic bacteria by altering the pH (Gibson and Roberfroid 1995).

MOS is believed to act by agglutination through the interaction of mannose-sensitive lectins that are located on the cell wall of the gram-negative bacteria. MOS prevent the colonization of harmful bacteria by competing for the attachment site in the digestive tract (Spring et al. 2000; Khare et al. 2018). Harmful bacteria adhere to mannans and pass the intestine without colonizing (Spring et al. 2000).

15.2.5 General Methodology Employed in Aquaculture Research Using Prebiotics

In order to determine the potential of food substances to be taken into consideration as a prebiotic component, various in vitro and in vivo experimentations are required to be performed. In vitro studies are widely used as compared to in vivo studies (Fig. 15.2).

The study of prebiotics can be carried out using different techniques based on three aspects as digestion, fermentation, and analysis. Digestion of various prebiotics can be examined by using different methodologies. In vitro digestion is widely used to study structural changes, digestibility in the upper gastric tract. The conditions provided in oral phase are NaCl and alpha-amylase at pH 7 for 5 min. The process of in vitro stomach is complex to imitate at gastric phase, and the last stage of prebiotic digestion can be tested in the small intestine of animal. In vivo digestion methodology estimates the measurement of a substrate in fecal matter (Khangwal and Shukla 2019)

The prebiotic fermentation by intestinal microflora can be checked out in the second step by the means of in vitro and in vivo fermentation. In batch culture system, pH-based batch culture system is utilized in which pH of fermentation system is maintained according to appropriate level (Saman et al. 2017). Continuous culture fermentation can be either single-staged or multi-staged which depends on the basis of fermentation. In vivo fermentation methodology is performed by collecting samples after treatment of prebiotics of model animals. Fecal samples are compiled after treatment of animal models with prebiotics. Human clinical

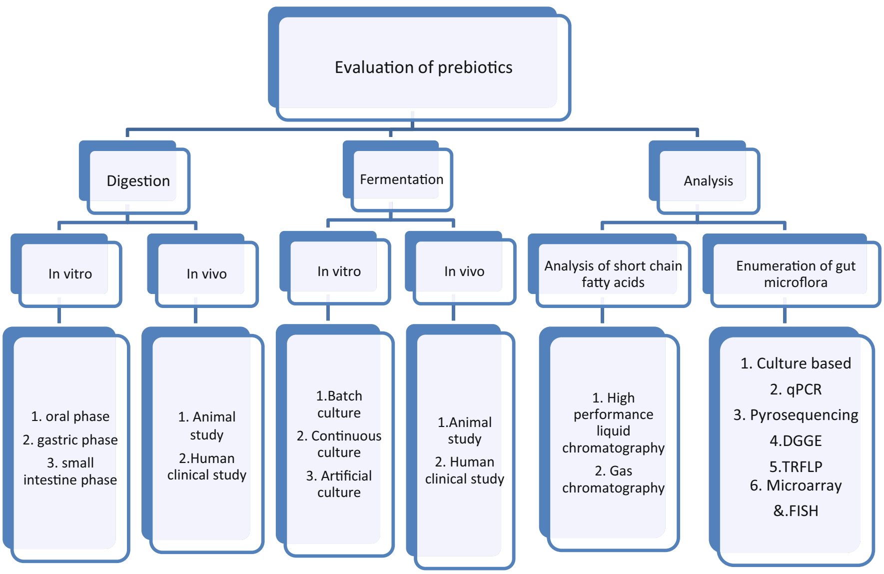


Fig. 15.2 In vivo and in vitro assessment of prebiotics and their components using various techniques (Khangwal and Shukla 2019)

studies are performed by either accumulating the breath air or fecal collection (de Oliveira et al. 2017).

Analysis of short-chain fatty acid is the final step of evaluation of prebiotics. These fatty acids are produced by gut microbiota during fermentation of carbohydrates. Prebiotics increase the gut microbiota and can be tested by HPLC and gas chromatography. Ion exchange and reverse phase columns are commonly used for screening. Culture-based techniques were used in the early centuries for detection of intestinal microflora. Later it moved to DNA-based methods which are culture independent to detect a particular amount of nucleic acids (DNA and RNA); the amount can be measured by real-time PCR. Pyrosequencing and 16s ribosomal RNA are common sequencing techniques used for sequencing of DNA and RNA of gut microbiota. The identification and comparison of gut microbiota diversity can be carried out by FISH, microarray, DGGE (denaturing gradient gel electrophoresis), and TRFLP (terminal restriction fragment length polymorphism) (Khangwal and Shukla 2019).

To study the effect of prebiotics in the animals, different methods and steps were used by different researchers.

15.2.5.1 Experimental Animals

Animals are allowed to adapt in the laboratory conditions before starting the experiment and further distributed into the groups according to the requirement of the experiment with control and treatment groups. For example, Nile tilapia (*Oreochromis niloticus*) are obtained from fish farms and maintained in aquarium tanks with adequate aeration under water internal power filter. Three groups of animals were used according to the supplementation of prebiotics including one control (without supplement) and two with different dosages of prebiotic supplements (Wakeel et al. 2019).

15.2.5.2 Preparation of Experimental Diets

Different mixtures of prebiotics with different dosages are prepared according to the experimental conditions. For example, to treat Nile tilapia (*Oreochromis niloticus*), three diets were formulated: the first group contained 0% of MOS as control, the second group contained 0.05% of MOS as feed+ adding MOS into water (12.5 mg/L), and in the third group MOS was added in water.

15.2.5.3 Feeding Trial

There can be one or more feeding trials followed by phases. These phases are comprised of treatment diet and control diet. Feed not consumed and fecal matter were removed for maintenance of good hygiene for experimental animals, and water quality needs to be measured regularly. The feeding trial for Nile tilapia (*Oreochromis niloticus*) includes twice daily feeding with an equal ratio at 9:00 h and 15:00 h for 6 weeks.

15.2.5.4 Performance Calculation

To observe the effect of prebiotics on the tissue, histological analysis is performed followed by qPCR and microarray for further analysis.

15.2.5.5 Data Analysis

To estimate the efficacy of prebiotic administration, the comparison of results is drawn between the treated and untreated groups for which the data obtained from different experiments are subjected to statistical tests such as ANOVA and t test.

15.3 Probiotics

Probiotic means the word of life which came from the Greek word *pro bios*. These include the use of live microorganisms that are used for the beneficial effect of human as well as animals. The term probiotics was initially considered as microbial products which might have some stimulatory impact on other microbes. The others were of the opinion that these are tissue extracts that can be supplemented with the feed of animals to restore the microbial balance of intestine. Recently, the scientists consider probiotics as food supplements of live microbes that can benefit the animal by improving the microbial balance if given in appropriate amount (FDA 2009). These can be consumed after the treatment of antibiotics because the antibiotics destroy the beneficial microflora along with the harmful ones present in the digestive tract. The steady consumptions of probiotics can help maintain the positive balance of microbial flora in the intestine.

These can be used as a regular dietary supplement or as a drug. Thus, these are categorized as either probiotics drug or probiotics food. In cases of dietary supplementation, there are no strict regulations required for safety and efficacy, but premarketing notifications are mandatory, but in cases of clinical application, proper safety measures need to be taken (Venugopalan et al. 2010). Hence, the application of probiotics as drug needs to undergo similar trials of safety efficacy at effective dose as any new drug.

15.3.1 Microbes Generally Considered Important as Probiotics and Their Mode of Action

The most commonly studied probiotics as food supplements as well as therapeutics include *Lactobacillus*, *Bifidobacterium* species, and others as mentioned in the table below (Kechagia et al. 2013) (Table 15.1).

With the extensive study of probiotics on human and land animal subjects, the potential of its use in the aquaculture industry seems significant as its optimum use can not only protect the animal from diseases but can also increase their survival resulting in higher revenue and food resource produce.

As per Pandiyan et al. (2013), the use of probiotics ensures better feed value with improvement in digestion due to probiotics enzymatic contribution and also has a

Table 15.1 Most important microbial species used as probiotics

Lactobacillus species	*L. acidophilus*, *L. casei*, *L. reuteri*, *L. crispatus*, *L. gasseri*, *L. rhamnosus*, *L. fermentum*
Other lactic acid bacteria	*Enterococcus faecalis, E. faecium, Lactococcus lactis, Leuconostoc mesenteroides*
Non-lactic acid bacteria	*Bacillus cereus, E. coli strain Nissle*
Bifidobacterium	The various *Bifidobacterium* species considered beneficial as probiotics include *B. adolescentis*, *B. bifidum*, *B. breve*, *B. lactis*, *B. animalis*
Yeast	*Saccharomyces cerevisiae, S. boulardii*

protective effect against different diseases due to its immune response enhancing, mutagenesis, and carcinogenesis prevention activity.

Probiotics are generally added to the rearing media and sometimes also added as food supplement. These probiotics can provide many benefits to the aquatic live-stock as their gut microflora is regularly replenished with the microbes from the water or food source. The probiotics added in the rearing medium can compete with the pathogens for target site. Some probiotics can also get established in the gut to provide longer benefits like immune stimulant. Others can even detoxify the water body, thus acting as bioremediating agents (Gatesoupe 1999).

The important properties which confer the probiotic with beneficial effect include the ability of the bacterium to adhere to cells and exclude pathogens by reducing their adherence to epithelium. These can even kill the pathogen by producing bacterial toxins (bacteriocins), acids, and even strong oxidant as hydrogen peroxide; nonetheless, these probiotics like *Lactobacillus* is considered safe as these do not invade the intestine, neither are they reported to cause cancer (Sobel 1999).

The yeast Saccharomyces. boulardii has been extensively studied for beneficial effect on human and animal subjects against acute and chronic gastrointestinal diseases in clinical trials. The major mechanisms of its action include antimicrobial activity against intestinal pathogens and maintenance of microbial homeostasis in intestine which modulates local and systemic immune responses (Mumy Karen et al. Mumy Karen et al. 2008). Moreover, the administration of *Saccharomyces* can also stabilize the gastrointestinal barrier along with proper functioning of digestion and nutrition absorption (Pothoulakis 2009).

The probiotics finds its use in therapeutics of human and animals due to the vast spectrum of benefits to the subjects. The exact mechanism of the probiotics activity cannot be singled out; some of the overlapping mechanisms have been suggested by various workers.

15.3.1.1 Regulation of Homeostasis in Intestine

In a healthy gut, there is presence of optimum balance of gut flora which is very important for the maintenance of intestinal homeostasis. In cases of antibiotics administration or any disease condition, this natural gut flora is altered which creates alteration in balance or homeostasis. This alteration can result in changes in the immune responses in the intestinal lumen as well as metabolism of epithelial cells. In such cases, the use of probiotics is recommended as these can regulate intestinal homeostasis by varying mechanisms.

The probiotics can reduce the adherence and invasion of pathogens on the intestinal epithelium as these use varying adhesion sites and can compete with the pathogen for the same. The pathogen can be either totally removed by the gut or find little surface to adhere (Bermudez-Brito et al. 2012.) Moreover, the probiotics reach the intestine live and can persist for long with regular multiplication. The coaggregation of probiotics in the intestine forms a normal and balanced type of intestinal microflora (Macfarlane and Cummings 1999). These can act also as an intestinal barrier and tight junction function by activating different intracellular signaling pathways. The proteins which are activated will induce expression of antimicrobial peptides like defensins, cathelicidins, as well as rearrangement of tight junction proteins. The signaling pathway also induces the expression of mucin which forms major component of mucous and forms a slimy barrier on the intestinal surface against the adherence and invasion of pathogens (La Fata et al. 2018). The luminal metabolism is also modulated by the probiotic produce of short-chain fatty acids like acetates and butyrates. These alterations can stimulate epithelial proliferation barrier (Markowiak-Kopeć and Śliżewska 2020).

As the probiotics colonize at the same sites occupied by the normal flora, it competes actively with pathogen in order to prevent its attachment and exclusion from host's intestine, urinogenital tract etc.

15.3.1.2 Antimicrobial Activity

Probiotics possess the ability to produce some active metabolites which have the ability to inhibit or kill the potential pathogen. The organic acids produced by probiotics lower the pH conditions in the intestinal and urinogenital region which strongly inhibit the growth of pathogens (Tharmaraj and Shah 2009; Ratsep 2014). The lactic acid-producing bacteria also produce hydrogen peroxide (H_2O_2) by the action enzymes like that of NADH oxidases and superoxide dismutase (Godi 2016) which hinder the growth of pathogens. Various bacteriocins or bacterial toxins are also produced by probiotics like enterolysins, enterocins, lactocins, and nisin (Arqués Juan et al. 2015; Šušković et al. 2010) which result in strong antimicrobial activity against susceptible pathogen. Bacteriocins are ribosome-coded short peptide; this proteinaceous antibiotic-like compounds are also produced by some probiotic strains which exert killing effect by pore formation in cell wall or interference in its synthesis (Pandiyan et al. 2013).

15.3.1.3 Immunomodulation

The general disease resistance in fishes is conferred by the innate immunity which is activated by normal microflora of the intestine. These innate immunity factors include the complements, lysozymes, transferrins, lectins pentraxins, and antimicrobial peptides. Moreover, the regulation of the innate immune response requires various factors like cytokines, interferon, TNFα (tumor necrosis factor), transforming growth factors, and chemokines (Gomez and Balcazar 2008).

The immunomodulatory impact of probiotics on rainbow trout was observed using oral dose of *Clostridium butyricum* which provided tolerance against vibriosis which increased the phagocytosis (Sakai et al. 1995). The blend of bacteria (*Bacillus* and *Vibrio* sp.) provides immunological strength in white shrimp juveniles by increasing phagocytosis and antibacterial activity which results in improved growth along with overall survival (Balcazar 2003).

15.3.2 Application of Probiotics in Aquaculture

Probiotics have been widely used in the treatment of various gastrointestinal (GI) and non-GI medical conditions. When probiotics are ingested orally, these passes through the abdominal system and attaches to the intestinal mucosa which intercept in the epithelial attachment of pathogenic bacteria (Scarpellini et al. 2008). *Bifidobacterium* and *Lactobacillus* bacteria produces different compounds like acetic acid, propionic acid, and lactic acid; these compounds are responsible to make the pH acidic and lead to inhibition of pathogenic bacteria.

15.3.2.1 Effect on Reproduction of Aquatic Species

The optimum breeding capacity of culturable organisms needs sufficient supplementation of nutrients particularly the optimum levels of proteins, lipids, and vitamins (C and E) (Izquierdo et al. 2001) along with probiotics (Ghosh et al. 2007). As per the study of Ghosh et al. (2007), the supplementation of *B. subtilis* as probiotics improved the reproductive performance of fish. This bacteria (*B. subtilis*) was initially obtained from the gut of carp and later studied for its impact on different ornamental fishes (*Poecilia* spp. and *Xiphophorus* spp.) at various doses for a long-term effect (1 year). The most significant results were obtained in terms of improved survival, productivity, as well as gonadosomatic index (GSI) at dose levels in the range of 10^6–10^8 cells/g of provided source of food. The GSI is a measure of gonadal mass with respect to the total body mass; thus, the improved GSI indicate accelerated growth of gonads or rapid attainment of puberty.

Other commercial probiotic which contains *Lactobacillus casei*, *L. acidophilus*, *Bifidobacterium thermophilum,* and *Enterococcus faecium* shows similar results with *X. helleri* which was carried out by Abasali and Mohamad (2010).

15.3.2.2 Improvement in Nutrient Digestion

The entire digestive process relies mainly on the presence of enzymes. These enzymes can be derived from host digestive system or by the probiotic organisms which are resident in the intestine. The optimum concentration of these enzymes ensures maximum extraction of nutrients from the food intake and results in better growth within less time interval. The probiotic organisms generally synthesize various enzymes like amylases, proteases, and lipases which are secreted extracellularly into the intestinal lumen. Moreover, these also provide growth factors like amino acids, fatty acids, and many vitamins which show beneficial effects on the digestion of water fauna (Balcázar et al. 2006). The probiotics supplementation of

Bacillus cereus strain E in diets of fish of Sparidae family *Dentex dentex* L shows increased fish growth (Hidalgo et al. 2006).

Some probiotics can also help in maturation as well as differentiation of the gastrointestinal tracts in juveniles like the probiotic yeast *Debaryomyces hansenii* HF1 which produces two important polyamines, spermine and spermidine, which are involved in the developmental process of intestines in mammals. Yeast is also involved in the improvement of digestion in sea bass larvae as this secretes enzymes like amylase and trypsin (Tovar et al. 2002).

The improvement in nutrient digestion is also aided with increase in the digestive surface which can also be achieved by use of probiotics like *Bacillus subtilis*. In a study conducted on swardtail and guppy fishes, the administration of *Bacillus subtilis* shows increase in the length and weight (Ghosh et al. 2008).

15.3.2.3 Growth Promotion

Many probiotics provide beneficial effect on digestion, absorption, or increase in animal appetite. All these factors provide better feed efficiency and higher growth rate in the treated group. Furthermore, indirect evidences of benefits have also been witnessed with improvement of live aquaculture feed production in presence of probiotics. High density growth of live food microalgae *Chaetoceros muelleri* and *Vibrio alginolyticus* C7b probiotics was observed which can be used to feed shrimps (Gomez-Gil et al. 2002). Good growth of Nile tilapia with dietary *Streptococcus* strain was observed in terms of increased levels of bodily components as crude protein and lipid along with overall improved weight in fishes (Lara-Flores et al. 2003). The growth and survival of ornamental fishes like swordtail and guppy also increase with probiotic supplementation with *Streptococcus* and *Bacillus subtilis* (Ghosh et al. 2008).

15.3.2.4 Inhibition of Pathogens

As antibiotics are utilized for longer duration in the field of aquaculture, this will give rise to resistance mechanism in microorganisms which causes disproportion in the gastrointestinal microbiota of aquatic species, which influences their well-being. Probiotic microorganisms have the bactericidal or bacteriostatic capacity with their produce of various substances, which pose negative impact on pathogenic microscopic organisms and hence do not let the opportunistic pathogens proliferate. For example, in probiotics-treated tilapia, *Oreochromis niloticus*, increment is observed in innate immune response, by production of different chemicals, for example, lysozyme action, neutrophile relocation, and bactericidal action, which improved the resistance of fish from the disease by *Edwardsiella tarda* (Taoka et al. 2006).

Epinephelus coioides which is one of the most traded aquaculture products has been tested for the effect of probiotics (*Bacillus subtilis* E20) against susceptibility to *Streptococcus* and *Iridovirus*. In comparison to the control, the probiotics administration promoted growth, enhanced immunity, and resistance against pathogen (Liu et al. 2012). *B. subtilis* E20 has also been tested on white shrimp larvae (*Litopenaeus vannamei*). The tested larvae provided evidences of growth acceleration, development, stress tolerance, as well as immune status when the probiotic was added in rearing water (Liu et al. 2010).

15.3.2.5 Improving Water Quality

The introduction of probiotics as food additive in water bodies also raises concerns about the extra introduction of microbes in natural or artificial rearing media. But surprisingly promising results have been obtained from various reports in terms of improvement in water quality parameters like dissolved oxygen, pH, temperature, ammonia, and hydrogen sulfide for the culture of shrimps and prawns (Chauhan and Singh 2019).

The excess organic matter in the water bodies are generally toxic as it raises the BOD (biological oxygen demand), thus the control of its concentration in water bodies is essential to maintain the optimum water quality for aquaculture. The administration of *Bacillus* spp. as probiotics has been reported to reduce the organic matter by its efficient conversion into other products as CO_2 which diffuses soon and incorporates into bacterial biomass along with surface slime (Mohapatra et al. 2013).

The nitrogen-based products like ammonia and nitrates also compromise the quality of water, which are removed by the administration of nitrifying bacteria to improve the quality of water (Mohapatra et al. 2013).

15.3.3 Commercial Products

For the manufacturing of probiotics, the item ought to satisfy and meet severe measures to be characterized as probiotic product which contain quality, security, and usefulness (Vankerckhoven et al. 2008). Various strains of microorganisms are utilized in the production of probiotic products which incorporate *Bifidobacterium*, *Lactobacillus*, *Streptococcus*, *Pediococcus*, and *Lactococcus* species. Most of these probiotics commercially available products are accessible in various forms in the market like sachet powder, case, and gum. Certain type of data is given by the provider on the product like microbial substance and their colony forming unit (CFU), manufacturing date, and expiry date. The quantity of utilization of probiotic products by people for different health-related issues has increased in recent times, as probiotics work to regulate host immunity against the pathogen present in the host and furthermore attack their colonizing population and also help in maintaining the stability of gut microflora (Patro et al. 2015).

At present, the production of lyophilized product has an advantage for storage and transport. Their reconstitution at optimum levels of hydration, temperature, and salt concentration certifies the feasibility of microbes (Muller et al. 2009). The health benefits incurred by the use of probiotics depends on many factors as, the ease to handle, store, reconstitute, and further viability and stability in the intestine of the host for longer duration (Irianto and Austin 2002).

Table 15.2 Some probiotics product used in aquaculture

Probiotic product name	Species	Activity
Biostart	*Bacillus* isolates	Rise in the viability and quality of pond-raised shrimp (Martínez Cruz et al. 2012)
Cernivet LBC and Toyocerin	*B. toyoi* isolates and *E. faecium* SF68 isolates	Decrease in death of the European eel (Martínez Cruz et al. 2012)
Biogen	*B. subtilis*	There is increase in the productivity by feed of *Oreochromis niloticus* (Haroun et al. 2006)
Combination strains	*C. butyricum B. subtilis, L. acidophilus, S. cerevisiae*	It increases the innate immunity parameters in tilapia (*Oreochromis niloticus)*, providing protection from *Edwardsiella tarda*-caused disease (Taoka et al. 2006)

15.3.4 Combinations of Strains

Generally, probiotics are applied singly or in combination with mutually compatible strains. It was studied that combinations of two or more cultures will be more helpful and efficient in comparison to single-strain probiotics, as multi-strain probiotics will increase the defense mechanism of organism against infectious diseases (Kesarcodi-Watson et al. 2012). The combination of two probiotics *L. acidophilus* and *B. subtilis* provides better results in Nile tilapia in terms of bactericidal activity and blood corpuscular concentration (hematocrit values) as compared to single strain. The growth and survival of *Labeo rohita* by the use of multi-strain probiotics enhanced fry and hatchling stages (Chauhan and Singh 2019) (Table 15.2).

15.3.5 General Methodology Employed in Aquaculture Research Using Probiotics

Strain identification: The correct identification of species is an essential step for the production of probiotic product. The currently used methods include 16s rDNA sequencing or DNA/DNA hybridization along with biochemical characterization to establish the recent taxonomic position of the probiotic organism (Gueimonde and Salminen 2006).

Physiological characteristics: The selection of specific substrate or enzymatic activity is a very important step for the relevant functional effects of strains. Other tests should also be performed like ability to hydrolyze bile salts (Lim et al. 2008) or to produce antimicrobial substances which is also considered important depending on the future application.

Tolerance to gastrointestinal conditions: The overall efficacy and effectivity of the probiotics include its successful reach, stability, and prolonged viability in the intestine of the host. To reach up to the intestinal lumen, it should possess the ability

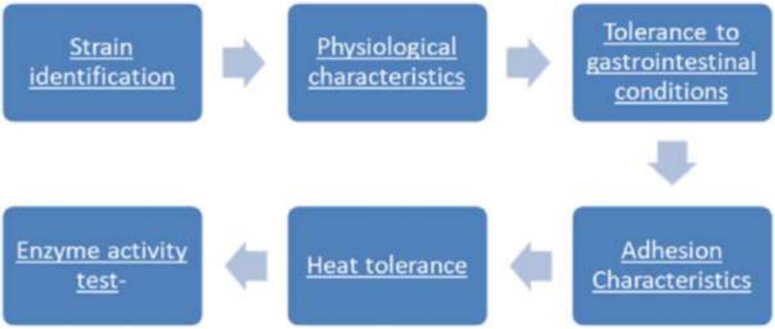


Fig. 15.3 General methodology employed in aquaculture research using probiotics

to tolerate acidic pH conditions in the stomach and bile salts in the intestine, although the tolerance to both the conditions is variable among bacteria (Drago et al. 2004).

Adhesion characteristics: Bacterial strain adhesion to the mucosa of the intestine is a significant rule for determination of probiotics. This assistance is to build the maintenance of a probiotic in the GI tract and put microbes and epithelial cells in proximity. Some profoundly adhesive microorganisms like *Bifidobacterium lactis* Bb12 and *L. rhamnosus* GG are viewed powerful for the treatment and anticipation of acute diarrhea (Gueimonde and Salminen 2006).

Heat tolerance: The isolated organisms like bacteria and yeast strains should also be inspected through an alternate procedure for heat tolerance. In a study, cultures were grown and maintained overnight in sterile broth (like MRS, YPD, and nutrient media) and heat treatment was given to culture cells (1–2 × 107 CFU/mL) for time intervals of 5 min, 10 min, 20 min, 30 min, and 60 min at three sets of temperature ranges viz 30 °C—80 °C—90 ¯C—100 °C, 37 °C—50 °C—60 °C—70 °C, and 30 °C—40 °C—52 °C (Kim et al. 2019).

Enzyme activity test: The microorganisms are resistant to different hydrolytic enzymes such as lysosomal activity which was evaluated using fluorescent-based microplate reader. For proteolytic activity test by using gelatin, 2% skimmed milk was used, with slight modifications in concentration. Similarly protease enzyme activity test is estimated by calculating the clear zone around the paper disks (Vijayaraghavan and Vincent 2013) (Fig. 15.3).

The probiotic products containing *Lactobacillus* species, for instance, *L. plantarum*, *L. rhamnosus*, *L. acidophilus*, *L. fermentum*, and *L. gasseri*, considered as a huge probiotics since their strain unequivocal properties are extremely useful for the well-being (Giraffa et al. 2010). Especially *L. plantarum* and *L. paracasei* were utilized as food supplements as a result of its high versatility towards acidic pH of stomach and bile salt emission in human and other vertebrates. *L. plantarum* and *L. paracasei* containing probiotics are applied in clinical treatment of various progressing and cardiovascular diseases, for instance, neurogenetic diseases, diabetes, hypertension, urinogenital multifaceted nature liver issues, etc.

Some probiotics organism secretes different compounds like bacteriocins and certain metabolites like lactic acid which help in the reduction of harmful pathogens and increase the probability of their destruction and highlighting their ability to modulate gut flora. So, the lactic acid-producing bacteria are "generally recognized as safe" (GRAS) (Cotter et al. 2005).

15.4 Synbiotics

The promising studies on the use of separate prebiotics and probiotics in improvement of growth, disease resistance, food efficiency, immunomodulation, etc. acted as a background to study the combined action of both. Such combinations are designated as synbiotics which act as the "health-enhancing functional gradients" forming a key factor for sustainable aquaculture (Cerezuela et al. 2011). In any such combination studies, there is possibility of either synergism or complementarity with the applicability depending upon the mode of combined action.

Gibson and Roberfroid (1995) stated that the use of the synbiotics concept may give the benefit of both pre- and probiotic on the growth of fish mainly due to the synergistic effect. Many scientists have worked to deduce the best formula for application of synbiotics on many animal systems. Most popular mode of applications with their beneficial effect on aquaculture has been summarized in the following text.

The synbiotics are broadly divided into complementary and synergistic synbiotics on the basis of the overall impact.

15.4.1 Complementary Synbiotics

Complementary synbiotics comprises both pre- and probiotics to generate the separate advantages when each is administered at an effective dose. Presently, many available synbiotics fall into this category.

15.4.2 Synergistic Synbiotics

Synergistic means "the sum of the total is greater than the sum of the parts," so when it comes to synergism, the combined effect of the probiotic and the prebiotic is greater than the sum of the separate effects. Synergistic synbiotics are the premium type of synbiotics, mainly designed by world-renowned gut health scientists so that the prebiotic component helps improve the function of the probiotic component (Fig. 15.4).

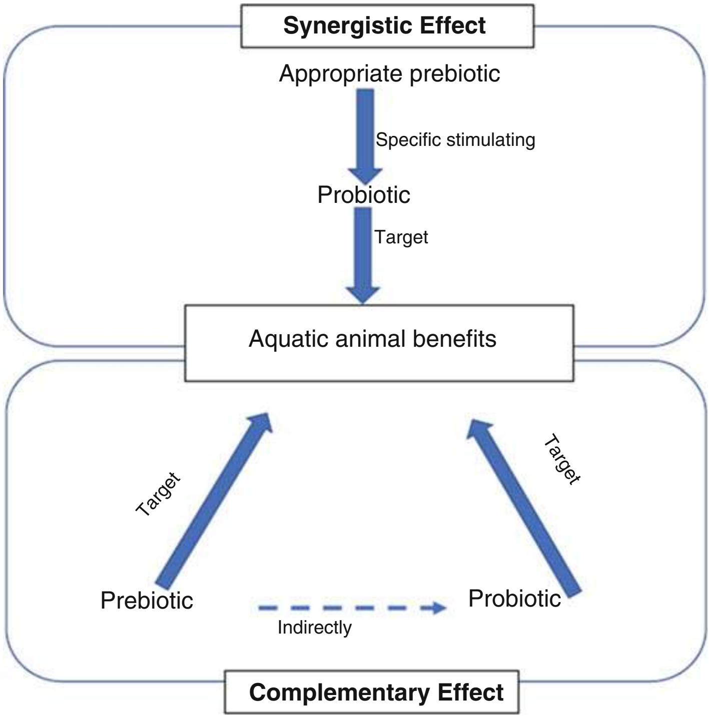


Fig 15.4 Synergistic and complementary effects of synbiotics (Kolida and Gibson 2011)

15.4.3 Sources and Safety Measures During Synbiotics Treatments

Synbiotics positively impact the host by increasing the survival and growth. This is accomplished by the establishment of live food supplements containing live microbes in the gut of host which can specifically stimulate the growth by triggering the metabolic processes of health-promoting bacteria.

The elements of synbiotics are generally obtained from various sources like fish mucus (Tapia-Paniagua et al. 2012), the gut of aquatic animals (Beck et al. 2015; Ramesh et al. 2015), collected cultures (Hjelm et al. 2004), as well as commercial products (Suzer et al. 2008). The other sources include natural aquatic ecosystem in which water or sediment acts as a good source of synbiotics components (Preetha et al. 2007), and some synbiotics can be separated from bio-flakes of microbe (Ferreira et al. 2015). Although the use of synbiotics provides considerable benefits on aquaculture produce, there seems less possibility that a single symbiotic composition would suffice all the desired properties, and practically, the chances of getting 100% results are not necessary. Moreover, caution needs to be taken before large-scale production and application as in many cases the effects observed during laboratory studies fail to repeat in field conditions (Kasarcodi and Watson et al 2008). The safety measure of synbiotic use should also be considered primarily with no obvious side effect in the applicable dose including non-harmful modified products, if any. The synbiotics should not contain any virulent or antibiotic resistance genes as these can be harmful to the host organism as well as the ecosystem.

15.4.4 Applications of Synbiotics on Aquaculture

15.4.4.1 Growth Improvement

The primary goal of aquaculture is using the best strategy of nourishment which will have a major effect on increasing net gain for commercial as well as environmental purposes. To improve growth, feed utilization, fulfilling of basic nutritional requirements, and to achieve the general health and stress resistance of the animals, some sort of supplements are required. Synbiotics can be applied through live microorganism supplementation or external bathing for promoting host welfare which helps in development, growth performance, proper feed utilization, as well as improvement of digestibility of aquatic organisms (Food and Agriculture Organization of the United Nations, World Health Organization 2002). Synbiotics are the live microorganism or ingredients which can be used singularly as probiotic and prebiotic as well as in combined form (synbiotic) to enhance the growth of fishes.

Group of fish Nile tilapia (*Oreochromis niloticus*) fed with supplemented diets (Biogen®) or (Pronifer®) for a period of 3 months showed that fishes grew better with a higher rate of growth performance as compared to control diet. According to Mehrim (2009) and Yassir et al. (2004), Nile tilapia (*O. niloticus*) fingerlings fed on a diet with yeast and 30% protein Biogen (synbiotic) generates the maximum growth with best feed conversion. Hence, it is proven that Biogen® is a good growth-stimulating additive in tilapia cultivation. In another study, hybrid striped bass were fed diets with synbiotic as GroBiotic or brewer's yeast for about 12 weeks which resulted in weight gain, better feed efficacy, and enhanced growth rate(Li and Gatlin 2005).

The improvement in overall growth of the animal can be attributed by the probiotics-induced enzymes and cofactors and the prebiotics induced better digestibility. The enzymes like protease, amylase, lipase, cofactors, and vitamins contributed by *Bacillus* spp. to the existing digestive enzymes improve the digestive process and hence improve growth in fishes and shrimps (Lee and Salminen 1995; Gatesoupe 1999; Gullian et al. 2004). Moreover, the use of prebiotics as spices and herbs such as onion, fenugreek, basil, and fennel to fish diets resulted in better protein digestion (Sakr et al. 2003) and feed efficiency which also contributes to growth improvement (Abd El-Rhman et al. 2009, Shalaby 2004). For growth parameters, largely all studies give a positive impact of synbiotics.

15.4.4.2 Improvement in Feed Efficiency

Synbiotics (probiotic and prebiotic) give positive impacts like increasing the survival rate with establishment of beneficial bacterial growing population in the gut of animal which improves digestion and absorption of the available food (Olsson et al. 1992). This results in higher growth rate or feed conversion.

The overall cost or expenditure incurred on the use of synbiotics is very well met with the reduction in feed cost and faster and better produce.

During the breakdown of synbiotics, several compounds are produced that can be used either by bacteria in the gut or host (Huynh et al. 2017). Besides this, the synbiotic ferments; essential amino acids such as lysine, phenylalanine, valine, tryptophan, and histidine; as well as non-essential amino acid such as vitamin A and K are also released (Rodríguez et al. 2011). Synbiotics serve as a source of exoenzymes which induce the host's digestive enzymes and thus can degrade more of the feed components and improve the absorptive ability of host which improves the WGR (weight gain ratio) or feed conversion.

15.4.4.3 Improvement in Immunity

Synbiotics are good immune stimulants, thus invigorating the immune systems of aqueous organisms, and enhancing the host protection against infection by viruses, fungi, bacteria, and parasites; apart from this, it also renders them far more resistant with prebiotic and probiotic either applied singly or combined (synbiotics). In ectothermic animals like fishes, the non-specific immune response acts as a major component of immunity. The fixed or mobile macrophages form the primary component of immune response which can phagocytize the pathogen and digest the same. The principle enzyme which is involved in this digestion is acid phosphatase (Attwood et al. 1996; Black et al. 1983). The increase in concentration of this enzyme is an indication of stronger immune response which has been reported by the use of *C. butyricum* to *Miichtys miicy* (Song et al. 2006). Another component of innate non-specific immunity is the lysozymes which are present in secretions and specifically act upon the peptidoglycans present in the bacterial cell wall, thus inhibiting its survival and sustenance and adherence on the internal body surfaces. The improvement in lysozyme activity has been reported with administration of synbiotics comprising FOS or/and MOS along with live *B. clausii* as feed additive in rainbow trout.The survival of juveniles and successful development to adult stage in aquaculture determines the overall production rate which is greatly hampered due to the impact of pathogen. In order to protect the juveniles, the study of the use of complex of *Bacillus* spp. and *Vibrio* spp. showed improvement in the immune resistance of *L. vannamei* juveniles for *V. harveyi-caused infection* (Moriarty 1990). Many components of synbiotics food additives like vibrio cells, lipopolysaccharides peptidoglycan, laminaria, yeast, and glucans have proved their immunostimulant activity in small-scale experimentation in aquaculture (Olsson et al. 1992). As per Mussatto and Mancilha (2007), the *Bifidobacteria* in the caecum colon produces immunostimulants which have inhibitory effect on the growth of putrefactive and pathogenic bacteria by fermenting some oligosaccharides.

The dietary incorporation of mannon oligosaccharides also referred as BioMas can significantly improve immune response by enhancing antioxidant activities and growth in many varieties of carp (Staykov et al. 2007; Zhou et al. 2004).

Major non-specific immune responses observed to improve after the administrations of synbiotics include the activation of alternative complement pathway, the macrophage-mediated phagocytic activity, and further digestion which improves respiratory burst; the improved superoxide dismutase activity is an indication of stress tolerance and even higher mucus production. In the case of fishes, the probiotic organism moves through the gut lining and multiplies to limited extent and soon perish, thus releasing the antigens. These antigens stimulate the immunity in the host. In the larvae and juveniles of aquatic invertebrates with weakly developed immunity, the immune protection might have been majorly not specific.

15.4.4.4 Biological Control Agents Against Diseases

Synbiotics were found to be useful not only as supplementary food ingredients for nutrient regeneration but also as biological controllers of fish diseases (Yasuda and Kitao 1980). The combination of prebiotic and probiotic (synbiotic) can be useful in the viable therapeutic modality in the treatment of gastrointestinal disease and hope of growth stimulation which affects the gut to treat with different chemotherapeutics to avoid bacterial infection and for reducing mortality ratio in fishes. The biological protection against mycobacterial infection is afforded by hybrid bass *Morone chrysops* after administration of 2% GroBiotic and brewer yeast (Li and Gatlin 2005). Similarly, significant protection from other pathogens like *Streptococcus iniae*, *Aeromonas hydrophila*, and *Mycobacterium marinum* have been observed after the supplementation of probiotics in wild and cultured hybrid striped bass (Overton and Roizen 2003).

15.4.4.5 Maintenance of Water Quality

In the aquaculture industry, there is a big concern of higher oxygen demand, acidic conditions due to the accumulation of lots of organic matter, and anaerobic mud in pond bottom sediments which poses adverse effect on organisms (Boyd and Gross 1998). To overcome the deterioration of soil and water quality in aquaculture systems and to avoid deposition of organic matter over time are the key concern. Research has proven that the use of synbiotics in aquaculture has enhanced the rate of degradation of organic matter, elevation in level of dissolved oxygen, elimination of undesirable waste products (nitrite ammonia, carbon dioxide, and sulfide), reduction in the proportion of blue-green algae, and increase aquatics production (Boyd and Gross 1998). The application of prebiotic and probiotic has proven to be effective in shrimp and fishes' hatcheries by outcompeting for pathogenic bacteria for nutrient and other resources, thus reducing the risk of disease and improving larval growth (Rengpipat et al. 1998).

Aquaculture products are vastly impacted with the use of synbiotics with overall improvement in hematological and biochemical properties like hematocrit value, total serum protein, high-density protein (HDL), triacylglycerols, cholesterol, low-density protein (LDL), albumin, globulin, and glucose in fishes (Ye et al. 2011; Mehrabi et al. 2012).

15.4.5 Synbiotics (Probiotic and Prebiotic) Administration Methods

After the successful research on the beneficial effects of synbiotics in pilot studies, the proper field application to ensure better feed conversion, growth rate, and overall profitability by use of correct concentration of feed additives is important. The benefits of synbiotics intake depend upon the mode of administration. The major modes are discussed below.

15.4.5.1 Combination

Synbiotics incorporate mixture of pro- and prebiotic, which depends on the standard of giving a combination of probiont over aquatic populations (Gibson and Roberfroid 1995). Use of combination of *Enterococcus faecalis* as probiotics and mannan oligosaccharide (MOS) as prebiotics exhibits higher food conversion ratio (FCR) in comparison to the individual applications (Rodriguez-Estrada et al. 2009). The European lobster even exhibited higher stress tolerance to low salinity conditions by use of combination of *Bacillus* spp. MOS (Daniels et al. 2015). A blend of probiotic with prebiotic, resistant energizer, or characteristic plant items has been utilized for development and endurance improvement (Van Hai and Fotedar 2009).

15.4.5.2 Encapsulation

This method involves encapsulation of live feed with synbiotics for enrichment. In this case, the synbiotics can survive or indeed even multiply on the live feed. Consequently, a live feed can transport synbiotic inside the host organism efficiently. The recent approach includes the enrichment of living food as Artemia, Rotifers, Copepods (Daniels et al. 2015; Gatesoupe 1991; Sun et al. 2013) with synbiotic. *Artemia nauplii* has been efficiently used as a vector for encapsulating the mixture of *Pseudomonas synxantha* and *P. aeruginosa* for *Western lord prawns*, *Penaeus latisulcatus* (Hai et al. 2010).

15.4.5.3 Water and Feed Additives

Prawns can be treated at any phase of life period. The mode of application can be as an addition to the water in which the animals are cultured or mixed with feed. The prawns can also be bathed in suspension of live bacteria (Smith and Davey 1993; Gram et al. 1999; Sakai 1999; Gram et al. 1999; Zhou et al. 2009; Cha et al. 2013).

15.4.5.4 Live and Dead Supplements

Live as well as dead synbiotic supplements are considered effective and important in aquaculture. Although the supplementation with live probiotic application induces improved expression of tumor necrotic factor, interferons and immunoglobins in comparison with the dead (Panigrahi et al. 2011). The inactivated or killed probiotic, as well as synbiotic preparations, appears as an alternative to the application of viable ones in the small aquaculture setup or natural aquatic environments.

15.4.5.5 Appropriate Dosage

To obtain the maximum benefit out of aquaculture, the optimum dosing of the symbiotic supplementation is important as both excessive and inadequate doses show inappropriate results (Panigrahi et al. 2004; Bagheri et al. 2008). The overdose can also suppress the non-specific immune response (Sakai 1999). A dietary enhancement with *L lactis* at 108 CFU g^{-1} improved the development rate, lysozyme, antiprotease, serum peroxidase, and blood respiratory burst in Japanese flounder (Heo et al. 2013). The appropriate dosing of synbiotic needs to be critically optimized as per the organism, species, developmental status, and the actual objective of application (Merrifield et al. 2010).

15.4.5.6 Time Duration

The total duration of symbiotic application also needs to be optimized prior to mass scale application. The time duration can vary widely ranging from days to months. The long-term administrations are generally considered unsafe for the well-being of the aquatic species as it can induce suppression of non-specific immune response (Merrifield et al. 2010). Moreover, the rate or frequency of synbiotic application is another factor of concern. As per the study conducted by Guo et al. (2009), the daily supplementation during the culture period gives better results as compared to the alternate ones. In case of cod larvae, the repeated application is required in place of single addition a day (Skjermo et al. 2015). As the live synbiotics get implanted into the gastrointestinal tract of the host, a short-term exposure during the active feeding stage is sufficient in providing prolonged protection from the pathogen even during the non-supplementation period (Balcázar et al. 2007).

15.4.6 General Methodology Employed in Aquaculture Research Using Synbiotics

Synbiotics have been shown to reduce carriage of an aquatic pathogen in a variety of different food ingredients. To avail the beneficial effects of synbiotics, different methods were used by scientists; a gist of the general methodology is described below.

15.4.6.1 Experimental Animals

The experimental animals should be acclimatized to the conditions for about 7–10 days or more before experimentation. All necessary conditions of water, feed, light, and water quality parameters need to be stabilized as per the specific animal requirements. All the experimentations on animals used for research should be primarily approved by the animal ethical committee.

15.4.6.2 Group Distribution

According to the desired experiments, animals are categorized into groups with one control group. In each group, the feed is set according to the experimental requirements like if we wish to do analyze cholesterol then diet should be cholesterol rich. Body weight of animal should be continuously checked and recorded by researcher thrice weekly in the feed supplementation phase based on the initial (before experiment started) and final (after the experiment) for each experimental group.

15.4.6.3 Synbiotic Preparation

Mixture of probiotic + prebiotic prepared as per the protocol. The time duration of the experiment and way of administration depend upon experimental conditions and the purpose of the study. Time duration for any experiment can be 3 weeks to 3 months and prolonged time duration. For instance the hyper-cholesterolemic study on Wistar rats, to estimate the impact of synbiotic supplementation was completed in 30 days (Alves et al. 2017).

15.4.6.4 Analysis

After the completion of each interval, the tissues of the animals are fixed in suitable fixative and studied for histopathological conditions to establish the positive, negative, or neutral impact on bodily tissues. The blood of animals collected and corpuscular values as well as plasma/serum analysis is performed. The parameter studied by various workers include estimation of total protein, albumin content, globin content, serum glucose and triglycerides, cholesterol apart from the histological studies.

15.4.6.5 Microbiome Analysis

The fecal microbiome can also be analyzed by 16S rRNA sequencing using oligonucleotide primers. The general measures of target mRNA can be determined utilizing the similar cycle limit or threshold (Ct) strategy portrayed by Pfaffl (2001).

15.4.6.6 Immunological Parameters

Lysozyme activity, alternative complement pathway activity, superoxide dismutase activity, phagocytic activity, and respiratory burst activity can also be measured after synbiotic application.

15.4.6.7 Statistical Analysis

The researchers apply various statistical tests like ANOVA, student's *t* test, and chi-square test as per the individual experiments to compare the results using statistical software.

15.4.7 Modes of Action of Synbiotic (Prebiotic and Probiotic)

The result of the administered synbiotic supplement depends upon which mode of action is applied. A fruitful alliance between beneficial and disease-causing microbe is the major purpose of using synbiotics as the constituents of intestinal and skin flora of aquatic organisms. Therefore, a synbiotic must possess certain essential features to qualify as a useful agent in culturable condition for aquaculture. These features

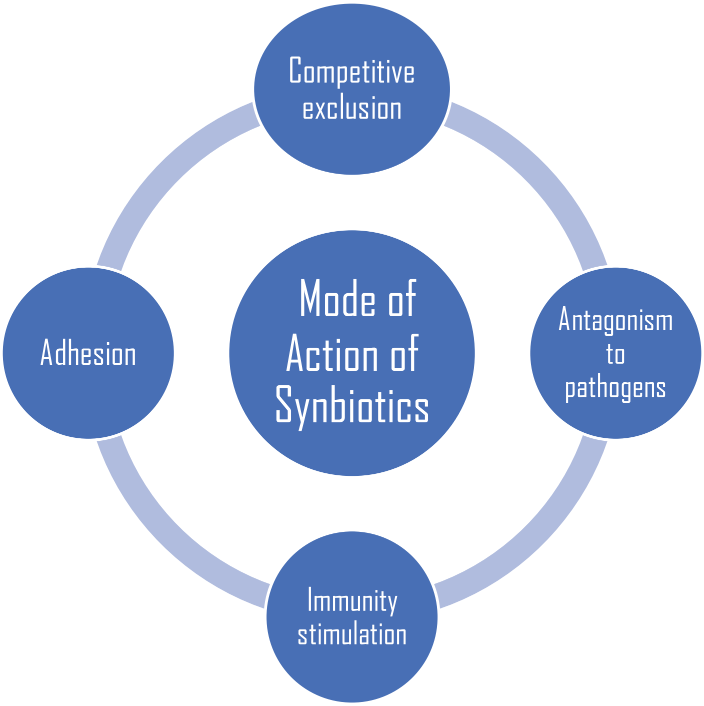


Fig. 15.5 Modes of action of synbiotics

include competitive exclusion, antagonism to pathogens, adhesion, and stimulation of immunity (Fig. 15.5).

The mode of competitive exclusion deals with the prevention of colonization of the undesirable bacteria in the intestine of host due to the competing action of the symbiotic organism which is established in the gut as feed additive. This competition can be at the level of attachment sites on mucosa or for available nutrients or even by the bactericidal activity of synbiotics (Gatesoupe 1989). The first step to establish in the intestine and attach to the surface is through the binding on the surface receptors, thus posing a strong competition to the pathogens (Montes et al 1993). The attachment to surface is aided by the binding with the enteric mucous lining of the intestinal wall (Olsson et al. 1992). The synbiotic bacteria like *Lactobacilli*, *Bifida* bacteria, and corn bacteria successfully colonize the gastrointestinal tract of many vertebrates including fishes (Freter 1992) with their firm attachment which prevents the loss by peristalsis (Beachey 1981).

In one experiment in vivo and in vitro, the adherence capability has been tested, and the results suggested that the potential synbiotic was capable to displace and suppress the pathogen by its ability to attach on mucus lining (Gatesoupe 1989). The pathogen inhibition is afforded by the use of synbiotics by many means which include prevention of its proliferation in the host due to competition, bactericidal activity of the synbiotics, and improved immune responses (Fuller 1992).

In aquaculture, synbiotics are most extensively used for prevention from the disease, for instance, like the vertebral column compression syndrome caused by *Flavobacterium psychrophilum*. To treat this disease, lactic acid bacteria (Villamil et al. 2002) or yeast (Siwicki et al. 1994) caused a decrease of this bacteria. Lactic acid bacteria produce compound bacteriocins that inhibit the growth of pathogenic microorganisms.

Bacterial hostility is a typical marvel that has a significant function in the harmony among useful and possibly pathogenic microorganisms (Balcazar et al. 2007). Antimicrobial metabolite production of bacteria is the key of antagonism test in vitro condition and is a frequently used way for screening of probiotic and prebiotic. Bacteria of the genus *Vibrio* spp. are part of the autochthonous flora of marine organisms and caused mortalities in shrimp larvae and juveniles (Rengpipat et al. 1998). A combination of probiotic and prebiotic (synbiotic) of *Bacillus* spp., *Lactobacillus* spp., and yeast had been used as their antagonistic effect to *Vibrio* spp. (Gullian et al. 2004).

15.5 Discussion and Conclusion

With the increased demand on aquaculture products as a good source of protein and nutrition to feed the growing population, greater emphasis has been laid on the improvement of feed efficiency, growth, and disease prevention. The decent use of prebiotics and probiotics separately holds promise to provide considerable beneficial effects, but greater benefits have been observed by the use of synbiotics. Strangely, the utilization of a mix of these parts exhibiting a synergistic impact might be considerably more effective in the increment of intestinal microbiota and security of the animal's well-being. The establishment of a strong disease prevention program, which includes synbiotic with good management practices, can raise aquatic organism production. Thus, the use of synbiotics (prebiotic + probiotic) seems compulsory to improve the nutritional health, prevention of diseases, reduction in stress, higher yield, etc.

The use of probiotic, prebiotic, and synbiotics is generally considered safe and does not have any side effects on the environmental components. The increase in disease resistance also reduces the use of antibiotics in aquaculture and thus helps in controlling the development of antibiotic-resistant strains. But greater scientific inputs are still required to meet all the challenges of aquaculture industry in the development and practical application of innovative technologies with promising breeders to obtain high yield and quality product. The advanced worldwide economy and solid market competitiveness and the contribution from scientists would provide support for the future betterment of these newly introduced technologies in the sustainable development of aquaculture.

References

Abasali H, Mohamad S (2010) Effect of dietary supplementation with probiotic on reproductive performance of female livebearing ornamental fish. Res J Anim Sci 4(4):103–107

Abd El-Rhman AM, Khattab YA, Shalaby AM (2009) Micrococcus luteus and Pseudomonas species as probiotics for promoting the growth performance and health of Nile tilapia, Oreochromis niloticus. Fish Shellfish Immunol 27(2):175–180

Akrami R, Abdolmajid H, Matinfar A et al (2009) Effect of dietary prebiotic inulin on growth performance, intestinal microflora, body composition and hematological parameters of juvenile beluga, Huso huso (Linnaeus, 1758). J World Aquacult Soc 40:771–779

Al-Salahy M (2002) Some physiological studies on the effect of onion and garlic juices on the fish, Clarias lazera. Fish Physiol Biochem 27:129–142. https://doi.org/10.1023/B:FISH.0000021913.60189.76

Alves MT, Ta NGH (2020) Models suggest pathogen risks to wild fish can be mitigated by acquired immunity in freshwater aquaculture systems. Sci Rep 10(1):7513. https://doi.org/10.1038/s41598-020-64023-2

Alves CC, Waitzberg DL, de Andrade LS et al (2017) Prebiotic and synbiotic modifications of beta oxidation and lipogenic gene expression after experimental hypercholesterolemia in rat liver. Front Microbiol 8:2010. https://doi.org/10.3389/fmicb.2017.02010

Amenyogbe E, Chen G, Wang Z et al (2020) The exploitation of probiotics, prebiotics and synbiotics in aquaculture: present study, limitations and future directions: a review. Aquacult Int 28(3):1017–1041. https://doi.org/10.1007/s10499-020-00509-0

Arqués Juan L, Rodríguez E, Langa S et al (2015) Antimicrobial activity of lactic acid bacteria in dairy products and gut: effect on pathogens. Biomed Res Int 584183:9. https://doi.org/10.1155/2015/584183

Attwood EM, Weich DJ, Oosthuizen JM (1996) The influence of carbon particles on the concentration of acid phosphatase and lysozyme enzymes within alveolar macrophages during the killing and degradation of Mycobacterium bovis. Tuber Lung Dis 77:341–347

Bagheri T, Hedayati SA, Yavari V et al (2008) Growth, survival and gut microbial load of rainbow trout (Oncorhynchus mykiss) fry given diet supplemented with probiotic during the two months of first feeding. Turk J Fish Aquat Sci 8(1):43–48

Balcazar JL (2003) Evaluation of probiotic bacterial strains in Litopenaeus vannamei: final report. National Center for Marine and Aquaculture Research, Guayaquil, Ecuador

Balcazar JL, Vendrell D, de Blas I, Ruiz-Zarzuela I, Gironés O, Múzquiz JL (2007) In vitro competitive adhesion and production of antagonistic compounds by lactic acid bacteria against fissh pathogens. Vet Microbiol 122(3–4):373–380

Balcázar JL, Blas ID, Ruiz-Z I et al (2006) The role of probiotics in aquaculture. Vet Microbiol 114(3-4):173–186

Balcázar JL, De Blas I, Ruiz-Zarzuela I et al (2007) Enhancement of the immune response and protection induced by probiotic lactic acid bacteria against furunculosis in rainbow trout (Oncorhynchus mykiss). FEMS Immunol Med Microbiol 51(1):185–193

Bayliss SC, Verner-Jeffreys DW, Bartie KL et al (2017) The promise of whole genome pathogen sequencing for the molecular epidemiology of emerging aquaculture pathogens. Front Microbiol 8:1–18. https://doi.org/10.3389/fmicb.2017.00121

Beachey EH (1981) Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surface. J Infect Dis 143(3):325–345. https://doi.org/10.1093/infdis/143.3.325

Beck BR, Kim D, Jeon J et al (2015) The effects of combined dietary probiotics Lactococcus lactis BFE920 and Lactobacillus plantarum FGL0001 on innate immunity and disease resistance in olive flounder (Paralichthys olivaceus). Fish Shellfish Immunol 42(1):177–183

Bentzon-Tilia M, Sonnenschein EC, Gram L (2016) Monitoring and managing microbes in aquaculture—Towards a sustainable industry. J Microbial Biotechnol 9(5):576–584. https://doi.org/10.1111/1751-7915.12392

Bermudez-Brito M, Plaza-Díaz J, Muñoz-Quezada S et al (2012) Probiotic mechanisms of action. Ann Nutr Metab 61:160–174. https://doi.org/10.1159/000342079

Black CM, Beaman BL, Donovan RM et al (1983) Effect of virulent and less virulent strains of Nocardia asteroides on acid phosphatase activity in alveolar and peritoneal macrophages maintained in vitro. J Infect Dis 148:117–124

Boyd CE, Gross A (1998) Use of probiotics for improving soil and water quality in aquaculture ponds. Adv Shrimp Biotechnol 101–105

Cerezuela R, Meseguer J, Esteban MA (2011) Current knowledge in synbiotic use for fish aquaculture: a review. J Aquacult Res Dev 1:1–7

Cha JH, Rahimnejad S, Yang SY et al (2013) Evaluations of Bacillus spp. as dietary additives on growth performance, innate immunity and disease resistance of olive flounder (Paralichthys olivaceus) against Streptococcus iniae and as water additives. Aquaculture 402:50–57

Chauhan A, Singh R (2019) Probiotics in aquaculture: a promising emerging alternative approach. Symbiosis 77:99–113. https://doi.org/10.1007/s13199-018-0580-1

Cotter PD, Hill C, Ross RP (2005) Bacteriocins: developing innate immunity for food. Nat Rev Microbiol 3(10):777–788. https://doi.org/10.1038/nrmicro1273

Daniels CL, Wills B, Ruiz-Perez M et al (2015) Development of sea based container culture for rearing European lobster (Homarus gammarus) around South West England. Aquaculture 448: 186–195

Davani-Davari D, Negahdaripour M, Karimzadeh I et al (2019) Prebiotics: definition, types, sources, mechanisms, and clinical applications. Foods 8(3):92. https://doi.org/10.3390/foods8030092

Demain AL, Vaishnav P (2009) Production of recombinant proteins by microbes and higher organisms. Biotechnol Adv 27(3):297–306. https://doi.org/10.1016/j.biotechadv.2009.01.008

de Oliveira GLV, Leite AZ, Higuchi BS et al (2017) Intestinal dysbiosis and probiotic applications in autoimmune diseases. Immunology 152(1):1–12. https://doi.org/10.1111/imm.12765

Dimitroglou A, Merrifield DL, Moate R et al (2009) Dietary mannan oligosaccharide supplementation modulates intestinal microbial ecology and improves gut morphology of rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Anim Sci 87(10):3226–3234

Dittmann KK, Rasmussen BB, Melchiorsen J et al (2020) Changes in the microbiome of mariculture feed organisms after treatment with a potentially probiotic strain of Phaeobacter inhibens. Appl Environ Microbiol 86(14):e00499–e00420. https://doi.org/10.1128/aem.00499-20

do Carmo MM, Walker JC, Novello D et al (2016) Polydextrose: physiological function, and effects on health. Nutrients 8(9):553. https://doi.org/10.3390/nu8090553

Drago L, De Vecchi E, Nicola L et al (2004) Microbiological evaluation of commercial probiotic products available in Italy. J Chemother 16(5):463–467. PMID: 15565913

Effendy JL, Simmons DL, Campbell GR et al (1997) The effect of aged garlic extract “Kyolic”, on the development of experimental atherosclerosis. Atherosclerosis 132:37–42

FAO Development of Marine and Inland Aquaculture. (2020). Retrieved 2 October 2020, from http://www.fao.org/3/B1363E01.html

Ferreira GS, Bolívar NC, Pereira SA et al (2015) Microbial biofloc as source of probiotic bacteria for the culture of Litopenaeusvannamei. Aquaculture 448:273–279

Food and Agriculture Organization of the United Nations, World Health Organization (2002) Guidelines for the evaluation of probiotics in food. In: Food and Agriculture Organization of the United Nations and World Health Organization Working Group Report. FAO/WHO, London

Food and Drug Administration (2009) Overview of dietary supplements [updated 2009 Oct 14; cited 2010 Mar 29]. http://www.fda.gov/Food/DietarySupplements/ConsumerInformation/ucm110417.htm

Freter M (1992) Factors affecting the microecology of the gut. In Probiotics. The scientific basis (Fuller R, ed), pp 111–145.

Fuller R (1992) History and development of probiotics. In: Probiotics. Springer, Dordrecht, pp 1–8

Ganguly S, Dora KC, Sarkar S et al (2013) Supplementation of prebiotics in fish feed: a review. Rev Fish Biol Fish 23:195–199

Gatesoupe FJ (1989) Further advances in the nutritional and antibacterial treatments of rotifers as food for turbot larvae, Scophthalmus maximus L. In: De Pauw N, Jaspers E, Ackefors H, Wilkins N (eds) Aquaculture – a biotechnology in progress. European Aquaculture Society, Bredene, pp 721–730

Gatesoupe FJ (1991) The effect of three strains of lactic bacteria on the production rate of rotifers, Brachionusplicatilis, and their dietary value for larval turbot, Scophthalmus maximus. Aquaculture 96(3-4):335–342

Gatesoupe FJ (1999) The use of probiotics in aquaculture. Aquaculture 180(1–2):147–165. https://doi.org/10.1016/S0044-8486(99)00187-8

Gentry RR, Alleway HK, Bishop MJ et al (2020) Exploring the potential for marine aquaculture to contribute to ecosystem services. Rev Aquacult 12(2):499–512. https://doi.org/10.1111/raq.12328

Ghosh S, Sinha A, Sahu C (2007) Effect of probiotic on reproductive performance in female livebearing ornamental fish. Aquac Res 38:518–526

Ghosh S, Sinha A, Sahu C (2008) Dietary probiotic supplementation on growth and health of live-bearing ornamental fishes. Aquacult Nutr 14(4):289–299

Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125(6):1401–1412. https://doi.org/10.1093/jn/125.6.1401

Gibson GR, Probert HM, Loo JV et al (2004) Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr Res Rev 17(2):259–275. https://doi.org/10.1079/NRR200479

Gibson GR, Scott KP, Rastall RA et al (2010) Dietary prebiotics: current status and new definition. Food Sci Technol Bull Funct Foods 7:1–19

Giraffa G, Chanishvili N, Widyastuti Y (2010) Importance of lactobacilli in food and feed biotechnology. Res Microbiol 161(6):480–487. https://doi.org/10.1016/j.resmic.2010.03.001. PMID:20302928

Godi NF (2016) Post harvest management of easily perishable horticultural crops using probiotics. Int J Adv Sci Eng Technol 4(2, Spl. Iss-2):75–77. IJASEAT-IRAJ-DOI-4681, http://www.iraj.in/journal/journal_file/journal_pdf/6-260-146588004675-77.pdf

Gomez-Gil B, Roque A, Velasco-Blanco G (2002) Culture of Vibrio alginolyticus C7b, a potential probiotic bacterium, with the microalga Chaetoceros muelleri. Aquaculture 211:43–48

Gomez GD, Balcazar JL (2008) A review on the interactions between gut microbiota and innate immunity of fish. FEMS Immun Med Microbiol 52:145–154

Gram L, Melchiorsen J, Spanggaard B et al (1999) Inhibition of Vibrio anguillarum by Pseudomonas fluorescens AH2, a possible probiotic treatment of fish. Appl Environ Microbiol 65(3):969–973

Gueimonde M, Salminen S (2006) New methods for selecting and evaluating probiotics. Dig Liver Dis 38 Suppl 2:S242-S247. https://doi.org/10.1016/S1590-8658(07)60003-6

Gullian M, Thompson F, Rodriguez J (2004) Selection of probiotic bacteria and study of their immunostimulatory effect in Penaeus vannamei. Aquaculture 233(1-4):1–14

Guo Z, Wang J, Yan L, Chen W, Liu XM, Zhang HP (2009) In vitro comparison of probiotic properties of Lactobacillus casei Zhang, a potential new probiotic, with selected probiotic strains. LWT Food Sci Technol 42(10):1640–1646

Gupta S, Lokesh J, Abdelhafiz Y et al (2019) Macroalga-derived alginate oligosaccharide alters intestinal bacteria of Atlantic Salmon. Front Microbiol 10:2037. https://doi.org/10.3389/fmicb.2019.02037

Haroun E, Goda A, Kabir M (2006) Effect of dietaryprobiotic Biogen supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia Oreochromis niloticus (L.). Aquacult Res 37(14):1473–1480

Heo WS, Kim YR, Kim EY et al (2013) Effects of dietary probiotic, Lactococcus lactis subsp. lactis I2, supplementation on the growth and immune response of olive flounder (Paralichthys olivaceus). Aquaculture 376:20–24

Hidalgo MC, Skalli A, Abellán E et al (2006) Dietary intake of probiotics and maslinic acid in juvenile dentex (*Dentex dentex* L.): effects on growth performance, survival and liver proteolytic activities. Aquacult Nutr 12(4):256–266

Hjelm M, Bergh O, Riaza A et al (2004) Selection and identification of autochthonous potential probiotic bacteria from turbot larvae (Scophthalmus maximus) rearing units. Syst Appl Microbiol 27(3):360

Hotchkiss AT, Olano Martin E, Grace WE et al (2003) Pectic oligosaccharides as prebiotics. In: Eggleston G, Cote G (eds) Oligosaccharides in food and agriculture [Chapter 5], ACS Symposium Series. American Chemical Society, Washington DC, pp 54–62

Huynh TG, Shiu YL Nguyen TP et al (2017). Current applications, selection, and possible mechanisms of actions of synbiotics in improving the growth and health status in aquaculture: a review. Fish Shellfish Immunol, 64, 367-382.

International Scientific Association for Prebiotics and Probiotics (2006) Annual meeting, Turku Finland. https://isappscience.org/for-scientists/resources/prebiotics/

Irianto A, Austin B (2002) Probiotics in aquaculture. J Fish Dis 25(11):633–642

Izquierdo M, Palacios H, Tacon A (2001) Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 197(1–4):25–42

Kechagia M, Basoulis D, Konstantopoulou S et al (2013) Health benefits of probiotics: a review. ISRN Nutr 2013:481651. https://doi.org/10.5402/2013/481651

Kesarcodi-Watson A, Kaspar H, Lategan MJ et al (2012) Performance of single and multi-strain probiotics during hatchery production of Greenshell™ mussel larvae, Perna canaliculus. Aquaculture 354:56–63

Khangwal I, Shukla P (2019) Prospecting prebiotics, innovative evaluation methods, and their health applications: a review. 3 Biotech 9(5):187. https://doi.org/10.1007/s13205-019-1716-6

Khare AG, Thorat A, Bhimte V et al (2018) Mechanism of action of prebiotic and probiotic. J Entomol Zool Stud 6(4):51–53

Kim J-A, Bayo J, Cha J et al (2019) Investigating the probiotic characteristics of four microbial strains with potential application in feed industry. PLoS One 14(6):e0218922. https://doi.org/10.1371/journal.pone.0218922

Kolida S, Gibson GR (2011) Synbiotics in health and disease. Annu Rev Food Sci Technol 2:373–393

La Fata G, Weber P, Mohajeri MH (2018) Probiotics and the gut immune system: indirect regulation. Probiotics Antimicrob Proteins 10(1):11–21. https://doi.org/10.1007/s12602-017-9322-6. PMID: 28861741; PMCID: PMC5801397

Lara-Flores M, Olvera-Novoa M, Guzmán-Méndez B, López-Madrid W (2003) Use of the bacteria streptococcus faecium and lactobacillus acidophilus, and the yeast Saccharomyces cerevisiae as growth promoters in Nile tilapia (Oreochromis niloticus). Aquaculture 216:193–201. https://doi.org/10.1016/S0044-8486(02)00277-6

Lee YK, Salminen S (1995) The coming of age of probiotics. Trends Food Sci Technol 6(7):241–245

Li P, Gatlin DM (2005) Dietary brewers yeast and the prebiotic GroBiotic AE influence growth performance, immune responses and resistance of hybrid striped bass (Morone chrysops × M. saxatilis) to Streptococcus iniae infection. Aquaculture 231:445–456

Lim MH, Lee OH, Chin JE et al (2008) Simultaneous degradation of phytic acid and starch by an industrial strain of Saccharomyces cerevisiae producing phytase and alpha-amylase. Biotechnol Lett 30:2125–2130. https://doi.org/10.1007/s10529-008-9799-x. Epub 2008 Jul 16. PMID: 18629438

Liu L, Yeh YY (2002) S-alk(en)yl cysteines of garlic inhibit cholesterol synthesis by deactivating HMG-CoA reductase in cultured rat hepatocytes. J Nutr 132(6):1129–1134. https://doi.org/10.1093/jn/132.6.1129

Liu KF, Chiu CH, Shiu YL et al (2010) Effects of the probiotic, Bacillus subtilis E20, on the survival, development, stress tolerance, and immune status of white shrimp, Litopenaeusvannamei larvae. Fish Shellfish Immunol 28(5-6):837–844. https://doi.org/10.1016/j.fsi.2010.01.012. Epub 2010 Feb 6. PMID: 20139006

Liu CH, Chiu C-H, Wang S-W, Cheng W (2012) Dietary administration of the probiotic, Bacillus subtilis E20, enhances the growth, innate immune responses and disease resistance of the grouper, Epinephelus coioides. Fish Shellfish Immunol 33(4):699–706., ISSN 1050-4648. https://doi.org/10.1016/j.fsi.2012.06.012

Lupton JR (2004) Microbial degradation products influence colon cancer risk: the butyrate controversy. J Nutr 134:479–482

Macfarlane GT, Cummings JH (1999) Probiotics and prebiotics: can regulating the activities of intestinal bacteria benefit health? BMJ 318(7189):999–1003. https://doi.org/10.1136/bmj.318.7189.999

Markowiak-Kopeć P, Śliżewska K (2020) The effect of probiotics on the production of short-chain fatty acids by human intestinal microbiome. Nutrients 12(4):1107. https://doi.org/10.3390/nu12041107. PMID: 32316181; PMCID: PMC7230973

Martínez Cruz P, Ibáñez AL, Monroy Hermosillo OA et al (2012) Use of probiotics in aquaculture. ISRN Microbiol 2012:916845. https://doi.org/10.5402/2012/916845

Meena DK, Das S, Kumar SC et al (2013) Beta-glucan: an ideal immunostimulant in aquaculture (a review). Fish Physiol Biochem 39:431–457

Mehrabi Z, Firouzbakhsh F, Jafarpour A (2012) Effects of dietary supplementation of synbiotic on growth performance, serum biochemical parameters and carcass composition in rainbow trout (Oncorhynchus mykiss) fingerlings. J Anim Physiol Anim Nutr 96(3):474–481

Mehrim A (2009) Effect of dietary supplementation of Biogen® (commercial probiotic) on monosex nile tilapia oreochromis niloticus under different stocking densities. J Fish Aquat Sci 4:261–273. https://doi.org/10.3923/jfas.2009.261.273

Merrifield DL, Dimitroglou A, Foey A et al (2010) The current status and future focus of probiotic and prebiotic applications for salmonids. Aquaculture 302(1-2):1–18

Mohapatra S, Chakraborty T, Kumar V et al (2013) Aquaculture and stress management: a review of probiotic intervention. J Anim Physiol Anim Nutr 97:405–430. https://doi.org/10.1111/j.1439-0396.2012.01301.x

Momeni-Moghaddam P, Keyvanshokooh S, Ziaei-Nejad S et al (2015) Effects of mannan oligosaccharide supplementation on growth, some immune responses and gut lactic acid bacteria of common carp (Cyprinus carpio) fingerlings. Vet Res Forum 6:239–244

Moriarty DJW (1990) Interactions of microorganisms and aquatic animals, particularly the nutritional role of the gut flora. In: Lésel R (ed) Microbiology in poecilotherms: proceedings of the international symposium on microbiology in Poecilotherms. Elsevier, Paris, pp 217–222

Mousavi E, Mohammadiazarm H, Mousavi SM et al (2016) Effects of inulin, savory and onion powders in diet of juveniles carp *Cyprinus carpio* (Linnaeus 1758) on gut microflora, immune response and blood biochemical parameters. Turk J Fish Aquat Sci 16:831–838

Muller JA, Ross RP, Fitzgeralk GF et al (2009) Manufacture of probiotic bacteria. In: Charalampopoulos D, Rastall B (eds) Prebiotics and probiotics science and technology. Springer, pp 125–759

Mumy Karen L, Chen X, Ciarán P et al (2008) Saccharomyces boulardii interferes with Shigella pathogenesis by postinvasion signaling events. Am J Physiol Gastrointest Liver Physiol 294(3): G599–G609. https://doi.org/10.1152/ajpgi.00391.2007. PMID - 18032477 4099 - https://journals.physiology.org/doi/abs/10.1152/ajpgi.00391.2007 4100

Munir MB, Hashim R, Abdul Manaf MS et al (2016) Dietary prebiotics and probiotics influence the growth performance, feed utilisation, and body indices of snakehead (Channa striata) fingerlings. Tropical Life Sci Res 27(2):111–125. https://doi.org/10.21315/tlsr2016.27.2.9

Mussatto SI, Mancilha IM (2007) Non-digestible oligosaccharides: a review. Carbohydr Polym 68(3):587–597

Olsson JC, Westerdahl ALLAN, Conway PL et al (1992) Intestinal colonization potential of turbot (Scophthalmus maximus)-and dab (Limanda limanda)-associated bacteria with inhibitory effects against Vibrio anguillarum. Appl Environ Microbiol 58(2):551–556

Overton J, Roizen M (2003) U.S. Patent Application No. 10/102,179

Pandiyan P, Balaraman D, Thirunavukkarasu R et al (2013) Probiotics in aquaculture. Drug Invent Today 5(1):55–59

Panigrahi A, Kiron V, Kobayashi T et al (2004) Immune responses in rainbow trout Oncorhynchus mykiss induced by a potential probiotic bacteria Lactobacillus rhamnosus JCM 1136. Vet Immunol Immunopathol 102(4):379–388

Panigrahi A, Viswanath K, Satoh S (2011) Real-time quantification of the immune gene expression in rainbow trout fed different forms of probiotic bacteria Lactobacillus rhamnosus. Aquacult Res 42(7):906–917

Passos HMR (2017) Effect of Levabon® Aquagrow E on the growth performance and immune response of European seabass (Dicentrarchus labrax) under stress conditions (Doctoral dissertation)

Patro J, Ramachandran P, Lewis J et al (2015) Development and utility of the FDA 'GutProbe' DNA microarray for identification, genotyping and metagenomic analysis of commercially available probiotics. J Appl Microbiol 118:1478–1488. https://doi.org/10.1111/jam.12795

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT–PCR. Nucleic Acids Res 29:e45

Pothoulakis C (2009) Review article: anti-inflammatory mechanisms of action of *Saccharomyces boulardii*. Aliment Pharmacol Ther 30:826–833. https://doi.org/10.1111/j.1365-2036.2009.04102.x

Preena PG, Raja Swaminathan T, Rejish Kumar VJ (2020a) Unravelling the menace: detection of antimicrobial resistance in aquaculture. Lett Appl Microbiol 71(1):26–38. https://doi.org/10.1111/lam.13292

Preena PG, Arathi D, Raj NS et al (2020b) Diversity of antimicrobial-resistant pathogens from a freshwater ornamental fish farm. Lett Appl Microbiol 71(1):108–116. https://doi.org/10.1111/lam.13231

Preetha R, Jayaprakash NS, Philip R et al (2007) Optimization of carbon and nitrogen sources and growth factors for the production of an aquaculture probiotic (Pseudomonas MCCB 103) using response surface methodology. J Appl Microbiol 102(4):1043–1051

Radecki SV, Yokoyama MT (1991) Intestinal bacteria and their influence on swine nutrition. In: Miller ER, Duane EU, Lewis AJ (eds) Swine nutrition. Butterworth—Heinemann, Boston, pp 439–447

Ramesh D, Vinothkanna A, Rai AK, Vignesh VS (2015) Isolation of potential probiotic Bacillus spp. and assessment of their subcellular components to induce immune responses in Labeorohita against Aeromonas hydrophila. Fish Shellfish Immunol 45(2):268–276

Ratsep M (2014) Effect of lactobacillus plantarum strains on clinical isolates of Clostridium difficile in vitro. J Probiot Health 02. https://doi.org/10.4172/2329-8901.1000119

Rengpipat S, Phianphak W, Piyatiratitivorakul S et al (1998) Effects of a probiotic bacterium on black tiger shrimp Penaeus monodon survival and growth. Aquaculture 167(3-4):301–313

Reverter M, Sarter S, Caruso D et al (2020) Aquaculture at the crossroads of global warming and antimicrobial resistance. Nat Commun 11(1):1870. https://doi.org/10.1038/s41467-020-15735-6

Ringø E, Olsen RE, Jensen I et al (2014) Application of vaccines and dietary supplements in aquaculture: possibilities and challenges. Rev Fish Biol Fish 24:1005–1033

Rodríguez E, Navarro-Villoslada F, Benito-Pena E et al (2011) Multiresidue determination of ultratrace levels of fluoroquinolone antimicrobials in drinking and aquaculture water samples by automated online molecularly imprinted solid phase extraction and liquid chromatography. Anal Chem 83(6):2046–2055

Rodriguez-Estrada U, Satoh S, Haga Y et al (2009) Effects of single and combined supplementation of Enterococcus faecalis, mannan oligosaccharide and polyhydroxybutyrate acid on growth performance and immune response of rainbow trout Oncorhynchus mykiss. Aquacult Sci 57(4): 609–617

Sakai M, Yoshida T, Atsuta S, Kobayashi M (1995) Enhancement of resistance to vibriosis in rainbow trout, Oncorhynchus mykiss (Walbaum), by oral administration of clostridium butyricum bacterin. J Fish Dis 18:187–190

Sakai M (1999) Current research status of fish immunostimulants. Aquaculture 172(1-2):63–92

Sakr M, Kendall R, Angus J et al (2003) Emergency nurse practitioners: a three part study in clinical and cost effectiveness. Emerg Med J 20(2):158–163

Saman P, Tuohy KM, Vázquez JA (2017) In vitro evaluation of prebiotic properties derived from rice bran obtained by debranning technology. Int J Food Sci Nutr 68:421–428

Sangeetha PT, Ramesh MN, Prapulla SG (2005) Recent trends in the microbial production, analysis and application of fructooligosaccharides. Trends Food Sci Technol 16:442–457

Santin E, Mairoka A, Macari M et al (2001) Performance and intestinal mucosa development of broiler chickens fed diets containing Saccharomyces cerevisiae cell wall. J Appl Poultry Res 10: 236–244

Scarpellini E, Cazzato A, Lauritano C et al (2008) Probiotics: which and when? Dig Dis 26:82–175

Scholz-Ahrens KE, Schaafsma G, van den Heuvel EG et al (2001) Effects of prebiotics on mineral metabolism. Am J Clin Nutr 73(2 Suppl):459S–464S. https://doi.org/10.1093/ajcn/73.2.459s

Sealey M, Barrowsb FTA, Hang A et al (2008) Evaluation of the ability of barley genotypes containing different amounts of β-glucan to alter growth and disease resistance of rainbow trout Oncorhynchus mykiss. Anim Feed Sci Technol 141:115–128. https://doi.org/10.1016/j.anifeedsci.2007.05.022

Senten J, Smith M, Engle C (2020) Impacts of COVID-19 on U.S. aquaculture, aquaponics, and allied businesses. J World Aquacult Soc 51(3):574–577. https://doi.org/10.1111/jwas.12715

Shalaby SW (2004) U.S. Patent No. 6,723,114. U.S. Patent and Trademark Office, Washington, DC

Siwicki AK, Anderson DP, Rumsey GL (1994) Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. Vet Immunol Immunopathol 41(1-2):125–139. https://doi.org/10.1016/0165-2427(94)90062-0

Skjermo J, Bakke I, Dahle SW, Vadstein O (2015) Probiotic strains introduced through live feed and rearing water have low colonizing success in developing Atlantic cod larvae. Aquaculture 438:17–23

Slavin J (2013) Fiber and prebiotics: mechanisms and health benefits. Nutrients 5(4):1417–1435. https://doi.org/10.3390/nu5041417

Small BC, Hardy RW, Tucker CS (2016) Enhancing fish performance in aquaculture. Anim Front 6(4):42–49. https://doi.org/10.2527/af.2016-0043

Smith PR, Davey S (1993) Evidence for the competitive exclusion of Aeromonas salmonicida from fish with stress-inducible furunculosis by a fluorescent pseudomonad. J Fish Dis 16(5):521–524

Sobel JD (1999) Is there a protective role for vaginal flora? Curr Infect Dis Rep 4:379–383. https://doi.org/10.1007/s11908-999-0045-z. PMID: 11095812

Soltanian Stuyven E, Cox E et al (2009) Beta-glucans as immunostimulant in vertebrates and invertebrates. Crit Rev Microbiol 35:109–138

Song M, Yun B, Moon JH et al (2006) Characterization of selected Lactobacillus strains for use as probiotics. Korean J Food Sci Anim Resour 35(4):551

Spring P, Wenk C, Dawson KA et al (2000) The effects of dietary mannaoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the cecae of Salmonella-challenged broiler chicks. Poult Sci 79:205–211

Srinivasan K (2005) Plant foods in the management of diabetes mellitus: spices as beneficial antidiabetic food adjuncts. Int J Food Sci Nutr 56(6):399–414. https://doi.org/10.1080/09637480500512872

Staykov Y, Spring P, Denev S, Sweetman J (2007) Effect of a mannan oligosaccharide on the growth performance and immune status of rainbow trout (Oncorhynchus mykiss). Aquacul Int 15(2):153–161

Su H, Liu S, Hu X et al (2017) Occurrence and temporal variation of antibiotic resistance genes (ARGs) in shrimp aquaculture: ARGs dissemination from farming source to reared organisms. Sci Total Environ 608:357–366. https://doi.org/10.1016/j.scitotenv.2017.07.040

Su X, Sutarlie L, Loh XJ (2020) Biosensors, and analytical technologies for aquaculture water quality. Research (Wash D C):8272705. https://doi.org/10.34133/2020/8272705

Sun YZ, Yang HL, Huang KP et al (2013) Application of autochthonous Bacillus bioencapsulated in copepod to grouper Epinephelus coioides larvae. Aquaculture 392:44–50

Šušković J, Kos B, Beganović J et al (2010) Antimicrobial activity—the most important property of probiotic and starter lactic acid bacteria. Food Technol Biotechnol 48:296–307

Suzer C, Çoban D, Kamaci HO et al (2008) Lactobacillus spp. bacteria as probiotics in gilthead sea bream (Sparus aurata, L.) larvae: effects on growth performance and digestive enzyme activities. Aquaculture 280(1-4):140–145

Taoka Y, Maeda H, Jo JY et al (2006) Growth, stress tolerance and non-specific immune response of Japanese flounder *Paralichthys olivaceus* to probiotics in a closed recirculating system. Fish Sci 72(2):310–321

Tapia-Paniagua ST, Díaz-Rosales P, León-Rubio JM et al (2012) Use of the probiotic Shewanella putrefaciens Pdp11 on the culture of Senegalese sole (Solea senegalensis, Kaup 1858) and gilthead seabream (Sparus aurata L.). Aquacult Int 20(6):1025–1039

Tharmaraj N, Shah NP (2009) Antimicrobial effects of probiotics against selected pathogenic and spoilage bacteria in cheese-based dips. Int Food Res J 16:261–276

Thornber K, Verner-Jeffreys D, Hinchliffe S et al (2020) Evaluating antimicrobial resistance in the global shrimp industry. Rev Aquacult 12(1):966–986. https://doi.org/10.1111/raq.12367

Toni M, Angiulli E, Malavasi S, Alleva E, Cioni C (2017) Variation in environmental parameters in research and aquaculture: effects on behaviour, physiology and cell biology of teleost fish. J Aquac Mar Biol 5(6):00137. https://doi.org/10.15406/jamb.2017.05.00137

Tovar D, Zambonino J, Cahu C et al (2002) Effect of live yeast incorporation in compound diet on digestive enzyme activity in sea bass (*Dicentrarchus labrax*) larvae. Aquaculture 204(1-2): 113–123

Tzounis X, Rodriguez-Mateos A, Vulevic J et al (2011) Prebiotic evaluation of cocoa-derived flavanols in healthy humans by using a randomized, controlled, double-blind, crossover intervention study. Am J Clin Nutr 93(1):62–72. https://doi.org/10.3945/ajcn.110.000075

Vankerckhoven V, Huys G, Vancanneyt M et al (2008) Biosafety assessment of probiotics used for human consumption: recommendations from the EU-PROSAFE project. Trends Food Sci Technol 19:102–114. https://doi.org/10.1016/j.tifs.2007.07.013

Van Hai N, Fotedar R (2009) Comparison of the effects of the prebiotics (Bio-Mos® and β-1, 3-D-glucan) and the customised probiotics (Pseudomonas synxantha and P. aeruginosa) on the culture of juvenile western king prawns (Penaeus latisulcatus Kishinouye, 1896). Aquaculture 289(3-4):310–316

Venugopalan V, Shriner K, Wong-Beringer A (2010) Regulatory oversight and safety of probiotic use. Emerg Infect Dis 16(11):1661–1665. https://doi.org/10.3201/eid1611.100574

Villamil L, Tafalla C, Figueras A et al (2002) Evaluation of immunomodulatory effects of lactic acid bacteria in turbot (scophthalmus maximus). Am Soc Microbiol 9(6):1318–1323. https://doi.org/10.1128/CDLI.9.6.1318-1323.2002

Vijayaraghavan P, Vincent SGP (2013) A simple method for the detection of protease activity on agar plates using bromo cresol green dye. J Biochem Technol 4:628–630

Wakeel A, Jan SA, Ullah I et al (2019) Solvent polarity mediates phytochemical yield and antioxidant capacity of Isatis tinctoria. Peer J 7(7857). https://doi.org/10.7717/peerj.7857

Watts JEM, Schreier HJ, Lanska L et al (2017) The rising tide of antimicrobial resistance in aquaculture: sources, sinks and solutions. Mar Drugs 15(6):158. https://doi.org/10.3390/md15060158

Yasuda K, Kitao T (1980) Bacterial flora in the digestive tract of prawns, Penaeus japonicus Bate. Aquaculture 19(3):229–234

Yassir E, Shalaby AME, Sharaf S, El-Marakby H, Rizkalla EH (2004) The physiological changes and growth performance of the Nile tilapia Oreochromis niloticus after feeding with Biogen as growth promoter. Egypt J Aquat Biol Fish 8:145–158
Ye JD, Wang K, Li FD et al (2011) Single or combined effects of fructo-and mannan oligosaccharide supplements and Bacillus clausii on the growth, feed utilization, body composition, digestive enzyme activity, innate immune response and lipid metabolism of the Japanese flounder Paralichthys olivaceus. Aquacult Nutr 17(4):e902–e911
Yun JW (1996) Fructooligosaccharides—occurrence, preparation, and application. Enzyme Microbiol Technol 19:107–117. https://doi.org/10.1016/0141-0229(95)00188-3
Zaman SA, Sarbini SR (2016) The potential of resistant starch as a prebiotic. Crit Rev Biotechnol 36(3):578–584. https://doi.org/10.3109/07388551.2014.993590
Zhou X, Li W, Kunkel HP et al (2004) A criterion for enhancing the giant magnetocaloric effect: (Ni–Mn–Ga)—a promising new system for magnetic refrigeration. J Phys Condens Matter 16(6):L39
Zhou XX, Wang YB, Li WF (2009) Effect of probiotic on larvae shrimp (Penaeus vannamei) based on water quality, survival rate and digestive enzyme activities. Aquaculture 287(3-4):349–353