Indu Pal Kaur *Editor-in-Chief*
Sandip V. Pawar
Praveen Rishi *Editors*

Probiotic Research in Therapeutics

Volume 2: Modulation of Gut Flora: Management of Inflammation and Infection Related Gut Etiology

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To Manjit, my husband who has always encouraged me in my professional journey and helped me enjoy finer things in life. To Bakul, Aagam, and Ramneek, my dear children who motivate me to outperform myself.

–Prof. Indu Pal Kaur

To my late father who always encouraged me and taught me to be independent and determined, To my loving mother for always supporting me, To Rohini, my wife for always motivating me, To Shreehan, my son for his endless love.

–Dr. Sandip V. Pawar

To Dharam Bir, my husband as well as to Valbha and Lavanya, my daughters for always encouraging me to have perfection in my academic life and supporting me to achieve my goals.

–Prof. Praveen Rishi

Foreword by J. V. Yakhmi

The saying attributed to Hippocrates, the Father of Medicine, that 'Let food be thy medicine, and let medicine be thy food' never felt more valid than now when we are challenged by a variety of life-style diseases. The relevance of holistic healing has increasingly been related, in recent years, to the gut microbiome, composed of bacteria, archaea, viruses, and eukaryotic microbes, all of which reside in our gut, and together have a strong potential to impact our physiology, both in health and in disease. When faced with a variety of diseases, our present-day knowledge lays emphasis on the importance of a healthy microbiome, not only limited to gut health but also to metabolic disorders, cancers, immunity, brain health, and skin health. Can we manipulate the gut microbiota by probiotic intervention towards disease prevention and treatment? That is precisely what is receiving the attention of a large number of scientists engaged in research on human health. The growing market interest in health benefits of probiotics has intensified research and investments in this area. With an overwhelmingly large number of new products based on probiotics on the shelves of the supermarkets and pharmacies, it can be inferred that the research in this area is at a very exciting stage. Though the intricate mechanisms involved in the importance of gut flora may require some basic scientific expertise, surfing through scientific claims on usefulness of probiotic therapy can catch the fancy of even a general reader.

I have known Prof. Indu Pal Kaur, Chief Editor of this book series, for the past 12 years and have been closely following her research interests which essentially hover around being a formulation scientist, be it for small and large molecules, phytochemicals and probiotics. I have noticed her deep interest in trying to complement the observational data compiled in the traditional system of medicine with scientific rationale from currently available information. I have myself discussed with her, several times, the human microbiome and its manipulations for useful therapeutic options. She has been active in the topic of probiotics for a long time, and had, in fact, published her first review on Potential Pharmaceutical Applications of Probiotics way back in 2002, which has been cited over 500 times till date. Her passion to bring probiotics into mainstream therapeutics is not limited only to the ailments of the gut, viz. inflammation, ulcers, and cancers, but is also aimed to extend it to other life-style diseases, such as depression, chronic fatigue syndrome, vaginal candidiasis, wound healing, and skin health.

The present ebook series, comprising five volumes, brings latest information and key insights on application of probiotics in cancer and immunological disorders, gut inflammation and infection, skin ailments, neurodegenerative disorders, and metabolic disorders. The contributing authors are recognized experts which ensure that each chapter affords a critical insight into the topic covered, with a review of current research, and a discussion on future directions in order to stimulate interest. Each volume itself covers a broad theme in detail by including chapters disseminating basic information in the field in such a manner that it would attract the attention of even a stray reader or intending consumers. Of course, the whole series of five volumes is designed with care so as to not only ignite the minds of graduating students for future research but also boost the confidence of health professionals, physicians, dieticians, nutritionists, and those practicing naturopathy by underlining the integrity of the data documented in the chapters of these volumes from well-established labs and groups. All in all, a very thoughtful compendium of probiotics research in therapeutics!

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Foreword by U. C. Banerjee

The probiotics have a long history of use and their benefits on human health are very well recognized although the scientific reasons were not well known earlier. Over the years, significant progress has been made in the areas of probiotic research with lots of scientific inputs. The probiotics are microorganisms which are used in foods, drugs, and nutritional supplements. A number of probiotic products are available in the market for health benefits to humans; the consumption of which has increased exponentially in the recent years. The gut microbiota in humans plays a vital role in health and disease and is influenced by the intake of probiotics. The endogenous beneficial gut microbes are also stimulated by prebiotics. The gut microbiota participates in activating immune system, absorbing essential nutrients, improving digestion, and inhibiting the growth of harmful pathogens. A number of research studies have been carried out to investigate the effect of probiotics either alone or in combination on immune functions, infections, and inflammatory conditions in humans. The researchers are currently engaged in exploring approaches to manipulate the human gut microbiota by probiotic-prebiotic combinations as a means of disease prevention and treatment.

The present e-book series entitled "Probiotic Research in Therapeutics", which is a compilation of five volumes that bring forth the purported benefits of probiotic therapy in a variety of diseases, is perceived and planned suitably by the editors. The book series encompasses the potential applications of probiotic therapy in modulation of gut flora, managing inflammation and infection, cancer and immunological disorders, neurodegenerative and metabolic disorders. With the state-of-the-art discussion on all the aspects of probiotic research from the individual contributors in the field of probiotic-prebiotic related research, the current book series provides an authoritative and timely overview of the field. The contents of all the chapters are informative and written by the experts in the field. Information compiled in the book series will be very much useful to the researchers, medical practitioners, and nutritionists. As a whole all the volumes are written in a very lucid manner so as

to make even a layman understand the concept. This is indeed an excellent addition to the existing knowledge on the subject.

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Preface

Studies on the diversity of human microbiota have dated back to the 1680s when Antonie van Leeuwenhoek studied and compared his oral and faecal microflora. However, the concept of *human microbiome* (a term coined by Joshua Lederberg in 2001) has intrigued scientists world over ever since its conceptualization. In this context, revelation of the magnanimity as well as the potential exhibited by human gut microbiome has stormed the scientific community ever since. In the last decade, we have witnessed breakneck paced research on various aspects of the gut microbiome and how it influences and plays a vital role in modulating key markers of health and disease. Various studies have documented its dynamic nature and hence continuous evolution with time, subject to various internal as well as external factors, thereby determining the equilibrium of the host, popularly referred to as homeostasis. In normal circumstances, microflora of the host and the gut are involved in a symbiotic association wherein the latter provides the bacteria with a nutrient rich environment for its survival as well as proliferation and the bacteria, in turn, aid in important functions of the host such as production of essential vitamins, absorption of iron, induction of immunity to confer protection from various pathogenic organisms along with directly competing with these harmful bacteria by production of various antimicrobial substances. This association is quintessential in determining the host health status and any deviation from this leads to a state of dysbiosis. Dysbiosis is characterized by a changed microflora wherein proliferation of pathogenic microbial population occurs resulting in a state of illness and a systemic state of inflammation is observed resulting in damage to the host. It is in such a state that replenishment of the beneficial bacterial population is necessitated and herein the role of probiotics holds immense significance.

Probiotics are defined as live bacteria which confer health benefits to host when administered in adequate amounts, as per WHO. They work by replenishing the gut with beneficial microbes, thereby providing holistic benefits to the host. The book elaborates on various properties of these friendly bacteria and their usefulness in a wide range of manifestations including inflammation, infection, and their relatively new role as upcoming therapeutic agents in cancers or as edible vaccines. The in-depth knowledge on various aspects of probiotics has been provided in a concise manner which is crucial and highly informative for anyone working in the field of probiotics.

We as scientists have always appreciated microorganisms and believed them to be smarter than humans. Is it a co-incidence that the world has been brought down on its feet by an invisible virus like SARS-CoV-2? To quote Neil deGrasse Tyson, 'Within one linear centimetre of your lower colon, there lives and works more bacteria (about 100 billion) than all humans who have ever been born. Yet many people continue to assert that it is us who are in charge of this world'. Let this serve as food for thought for the scientific community and thereby ponder upon the importance of living in harmony with the microbes.

Chandigarh, India Indu Pal Kaur
Chandigarh, India Sandip V. Pawar
Chandigarh, India Praveen Rishi

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About the Editors

Indu Pal Kaur presently the Chairperson and Professor of Pharmaceutics at the University Institute of Pharmaceutical Sciences, Panjab University, has more than 25 years of teaching and research experience. Her research forte is enhancing bioperformance of small and large biomolecules including probiotics using active-tailored delivery systems. Emphasis of her work lies on clinical translation of her work as evidenced by the transfer of three technologies from her lab to the Industry. She has been granted six Indian and one US patents and has 19 patent applications in the pipeline. She has >135 high-impact publications and 17 book chapters to her credit and falls in the top 2% of highly cited scientists in the world. A book volume on 'Nanobiomedicine' and special issues of journals 'Current Pharmaceutical Design' and 'Pharmaceutical Nanotechnology' have been edited by her. She has received funding to the tune of 72.5 million INR from Government agencies and has industrial consultancies amounting to 15.3 million INR. She was awarded the very prestigious 'Fulbright-Fellowship (FNAPE)' by USA, and was a visiting faculty at Rutgers State University, New Jersey, USA for 6 months in 2017–2018. The Organizers of Pharmaceutical Producers of India (OPPI) bestowed upon her 'The best Women Scientist Award-2018'. She was also awarded Researcher of the Year Award-2019, BRIC Idea Exposition Award-2019, BRIC Technology Exposition Award and Tynor Leadership Award for Innovation-2020.

Sandip V. Pawar is currently working as a UGC-Assistant Professor at the University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India since June 2018. Over the past 10 years, he got opportunities to work at some of the best industrial and research institutions in Canada and India. Dr. Pawar obtained his B. Pharm from University of Pune, India, M. Tech—National Institute of Pharmaceutical Education and Research, Punjab, India. He has worked previously as a Research Scientist—Bioplus Lifesciences (VB Medicare), Hosur, India after completing his M. Tech. Dr. Sandip completed his Ph.D. in bioprocess technology from the Institute of Chemical Technology, Mumbai. He was awarded with prestigious MITACS postdoctoral research fellowship at the University of British Columbia, Canada in August 2014. At UBC Vancouver, he worked on multidisciplinary research projects in areas of metagenomics, synthetic biology, and metabolic engineering for development of pharmaceuticals and biotechnological products in

collaboration with industry partners, viz., MetaMixis Inc., and InMed Pharmaceuticals. His primary area of research is Pharmaceutical Biotechnology. He has published 21 high-impact publications and filed one Indian and one US patents.

Praveen Rishi presently a Professor, Department of Microbiology, Panjab University, Chandigarh, has been working in the field of Medical Microbiology for the last several years. ln view of the emerging drug resistance in pathogens, her major research focus has been on the development of various biotherapeutics including antimicrobial peptides and probiotics. She has filed six patents and has one technology transfer to her credit. She has published more than 140 research publications in International journals of repute. She has carried out several research projects successfully, funded by various National agencies including ICMR and DST. In view of her contribution in several areas of Microbiology, she has been the recipient of DBT Overseas Associateship and INSA Visiting fellowship and has also been awarded prestigious National awards such as Prof SR Vyas Memorial Award by the Association of Microbiologists of India (AMI) and Dr. Y.S. Narayana Rao Award by the Indian Council of Medical Research (ICMR), Government of India. She was also elected as the Fellow of the Academy of Microbiological Sciences (FAMSc) and Fellow of the Indian Association of Biomedical Scientists (FABMS).

Gut Bacterial Dysbiosis and Its Clinical Implications

1

Ann Catherine Archer

Abstract

The gut microbiota as it is collectively known, functions as an organ of the human body influencing various metabolic, immunologic and neurologic activities. Our understanding of this microbial community has drastically increased in the past decade owing to a burst in 'omics' sciences. A healthy microbiome is essential for energy harvest and normal functioning of other body processes beyond the gut. However, perturbances in the gut microbiota referred to as 'dysbiosis' are said to hamper the homeostatic condition and are implicated in the development of a number of diseases such as obesity, metabolic syndrome, diabetes, inflammation, etc. A number of factors such as antibiotic misuse, dietary lifestyle, etc. are said to lead to bacterial dysbiosis. Since the gut microbial community is largely composed of bacteria, understanding the composition, factors influencing dysbiosis and its implications in several diseases is important. Thus, this chapter focuses on highlighting the importance of the gut microbiota, factors responsible for dysbiosis, effects of dysbiosis leading to several conditions as well as scope for normalizing the dysbiotic state.

Keywords

Gut microbiota · Dysbiosis · Inflammatory bowel disease · Obesity · Diabetes · NAFLD · Aging · Adverse pregnancy outcomes · Probiotics · Prebiotics

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1.1 Introduction

The human microbiome is a collective term for microorganisms such as bacteria, fungi, protozoa, viruses, etc. in symbiotic relationship with the human body (Ipci et al. 2016). Microorganisms harbouring our body outnumber the human cells by a ratio of 10:1. These microorganisms occupy different niches of the body and perform specific functions beneficial to the host. The total number of genes of all microbes in the microbiome is about 200 fold that of the human genome and weighs around 2 kgs. A major part of these microorganisms are found living in the gut, i.e. the gastrointestinal tract (GIT) especially the large intestine. An adult human GIT is composed of 10^{14} bacterial cells harbouring more than 1000 bacterial species. Bacteria which are the most widely studied organisms of the gut microbiome are commensal and perform several metabolic, physiological and immunological functions including digestion and absorption of food, development and regulation of immune system, prevention of diseases, maintenance of homeostasis, and production of vitamins such as vitamin B12, riboflavin, vitamin K, etc. These bacteria are hence considered not invaders but beneficial colonizers, part of the human evolution since inception (Thursby and Juge 2017). However, some bacteria are potentially harmful to the human body and may cause disease. In healthy condition, homeostasis of these bacterial flora is maintained by regulation and cross-talk between the host and gut microbiota preventing an overgrowth of harmful or pathogenic bacteria. This homeostatic balance is essential for maintenance of host health. However, changes in the microbial composition of the GIT can lead to imbalance between the commensal or beneficial flora and pathogenic bacteria making the gut highly susceptible to pathogen invasion and assault. This perturbation of the GIT equilibrium is termed as dysbiosis. Hence, dysbiosis is defined as imbalance of the gut microbial composition, distribution or metabolic activity affecting the microbial homeostasis (Bien et al. 2013). In general, dysbiotic state is characterized by reduction of beneficial organisms, overgrowth of pathogenic organisms and changes in overall microbial diversity. It is now known that microorganisms engage in cross-talk with the host and influence several processes in the gut and beyond. Hence, dysbiosis of this microbial niche is implicated in several diseases such as obesity, diabetes mellitus, inflammatory bowel disease (IBD), colorectal cancer, allergy, etc. Before we delve into the clinical implications of dysbiosis, it is essential to know the colonization and development of gut microbiome.

1.2 Development of Gut Microbiota

Earlier it was believed that the human body specifically the gut is colonized at the time of birth. However, recent studies suggest that initial colonization occurs in the womb of the mother in utero from various sources such as the GIT, oral cavity and skin of the mother. Evidences of microbes in placenta and amniotic fluid were reported by metagenomics and metabolic profiles of samples from pregnant women (Aagaard et al. 2014). At birth, the GIT is rapidly colonized by a wide

array of microorganisms which is dependent on various factors such as gestational age, mode of delivery (vaginal or caesarean), diet (breast fed or infant formula fed), hygiene and antibiotic treatment. Facultative anaerobes are the first colonizers which pave the way for colonization of strict anaerobes such as *Bacteroides*, *Clostridium* and *Bifidobacterium* spp. The intestinal microbiota initially harbours a low diversity of microorganisms belonging to phyla Proteobacteria and Actinobacteria gradually changing to a more diverse flora dominated by Firmicutes and Bacteroidetes. By the age of 3 years, the microbiota of the infant possesses a distinct microbial profile and resembles that of an adult with respect to composition and diversity. Thus, the first 3 years of life are crucial for the growth and development of the child. The microbiota is essential for neuro- and immune-development and hence disturbance of the microbiota composition in early years of life has long-term effects on the health and development of the host (Round and Mazmanian 2009; Koenig et al. 2011; Rodriguez et al. 2015). The GIT microbiota reaches a stable composition at adulthood although changes occur depending on several environmental factors.

1.3 Composition of Gut Microbiota and Factors Causing Dysbiosis

A wide number of bacterial species occur in the GIT. In the past, these species were identified by conventional labour intensive culture techniques, where faecal samples collected from different healthy individuals were collected and directly plated on artificial media (Moore and Holdeman 1974). These techniques have now become outdated and redundant as they are time consuming, labour intensive and results obtained are not precise. Nowadays, culture independent techniques such as sequencing have replaced conventional methods and also provide a better understanding of the microorganisms present in the gut. The 16S rRNA (ribosomal subunit gene) particularly has been widely applied for the characterization of bacterial communities in a variety of niches such as the endogenous human microbiome and host free communities like soil and ocean ecosystems. The 16S rRNA gene is ubiquitously present in all prokaryotic organisms and comprises areas which are highly conserved and variable regions which can be used to differentiate between taxa. Previous reports usually studied bacterial communities by sequencing the entire 16S rRNA gene. However, the entire array of bacterial diversity was not achieved due to limitations in depth of sequencing. With the advent of parallel sequencing techniques which produce short reads, nowadays sequencing of only short sub-regions of the gene is undertaken at greater depth. The second generation sequencing techniques encompassing large scale sequencing such as pyrosequencing provides up to 500 bp longer reads. However, the sequencing read length has increased with the introduction of advanced sequencing using Illumina MiSEQ and HiSEQ and provides higher and cheaper output. Furthermore, whole genome shotgun metagenomics studies are the techniques of today which provide reliable measures of microbiota diversity and composition with high resolution and sensitivity (Poretsky et al. 2014).

Majority of the bacterial species present in the GIT belong to two major phyla, namely Firmicutes and Bacteroidetes while others belonging to Actinobacteria, Proteobacteria, Fusobacteria, Verrucomicrobia and Spirochaetae occur in smaller amounts. While the dominating phyla are almost constantly maintained among individuals, each individual harbours a unique composition of microbial species. Based on these, three distinct enterotypes (clusters) of microbiota have been identified in humans and these enterotypes are independent of age, gender or geographical location (Arumugam et al. 2011). Dysbiosis can isolate specific cluster of enterotype which are found to be associated with disease such as inflammation, atherosclerosis, non-alcoholic fatty liver disease (Arumugam et al. 2011; Ahmad et al. 2019; Zhao et al. 2019). Factors such as diet, antibiotic use, stress, etc. can cause alterations in the indigenous enterotype. Use of antibiotics in infants is said to disturb the development of the normal microbiome and may have long-term effects. Antibiotics in addition to pathogens also kill beneficial flora thus making the host more susceptible to opportunistic infections. Stress on the other hand can result in the reduction of beneficial microorganisms like *Lactobacillus* and Bifidobacteria and simultaneously cause the overgrowth of pathogenic microorganisms. The effects of stress induced dysbiosis have been observed in several studies (De Palma et al. 2016; Dodiya et al. 2019). Diet is one of the major factors which influences the composition of the gut microbiota and could act as both cause of dysbiosis and modulator of dysbiosis. The effect of diet will be discussed in a separate section in this chapter. Hence, identifying the cause and dietary components which beneficially modulate dysbiosis can help prevent dysbiosis related diseases.

1.4 Clinical Implications of Dysbiosis

Dysbiosis of the GIT microbiota which perform several functions essential for the host can lead to several diseases or clinical implications. These are related to changes in microbial diversity, metabolic and immune functions which have localized implications in the GIT and most often affect the host beyond the boundaries of the GIT. Some of the diseases associated with dysbiosis of the gut microbiota along with changes in microflora characteristic of each disease are summarized in Fig. 1.1.

1.4.1 Inflammatory Bowel Disease (IBD)

IBD is a multifactorial disease which includes Crohn's disease (CD) and ulcerative colitis (UC) characterized by genetic impairment along with disrupted intestinal barrier function. Three particular pathogens are said to be associated with the pathogenesis of IBD, namely *Mycobacterium avium paratuberculosis,* adherent invasive *Escherichia coli* (AIEC), and *Clostridium difficile. M. avium paratuberculosis* was initially implicated as a causal agent of CD, AIEC was found associated with acute phase of IBD and increased inflammatory response while *Clostridium difficile* has been found in patients suffering from UC (Clayton

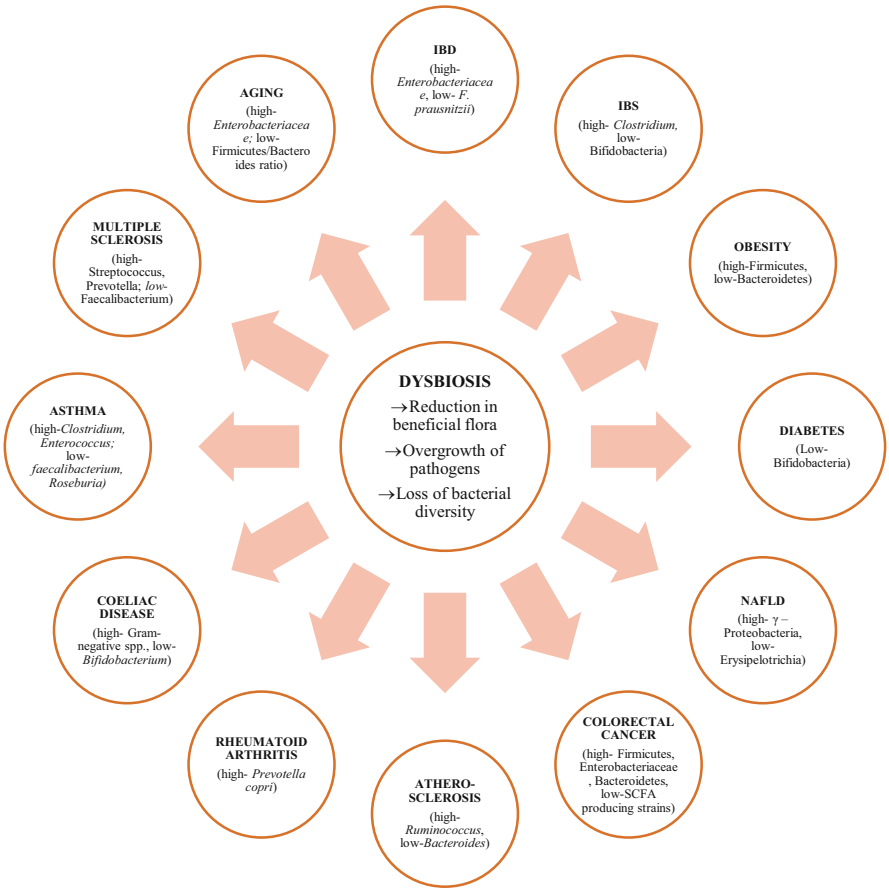

Fig. 1.1 Clinical implications of dysbiosis. Gastrointestinal and systemic diseases as a result or consequence of dysbiosis and the major microbial species associated with each disease as discussed in this chapter are summarized in this diagram

et al. 2009). However, studies of these bacteria being the causal agent of IBD are not sufficiently proved. In fact, it is thought that IBD may be caused by dysbiosis of the gut microbiota but whether dysbiosis is the cause of the disease or an effect is still unclear. Dysbiosis in IBD shows a characteristic decrease of Firmicutes and Bacteroides which are said to be abundant in otherwise normal flora (Hedin et al. 2014; Peyrottes et al. 2019). There is a significant increase of *Enterobacteriaceae* members in CD (Li et al. 2015; Halfvarson et al. 2017; Brusaferro et al. 2019). In particular, an increase in *Ruminococcus gnavus* is seen with a decrease in *Faecalibacterium prausnitzii*, *Dialister invisus*, *Bifidobacterium adolescentis*, and an unknown *Clostridium* spp. (Joossens et al. 2011). Intestinal dysbiosis in CD is also characterized by enhanced intestinal permeability. Intestinal permeability is an important feature of the gastrointestinal barrier integrity influenced by the gut

microbiota as well as the mucosal immunity (Bischoff et al. 2014). Barrier integrity is essential to protect the GIT from adhesion and penetration of intraluminal antigens into the underlying lamina propria, hence, maintaining a healthy GIT. Gut microbiota play a critical role in maintaining the gut barrier integrity. Dysbiotic shifts of the gut microbial species characteristic of CD such as reduction of *Faecalibacterium prausnitzii* and increase of *Enterobacteriaceae* members could result in either improvement or loss of gut epithelial barrier function (von Martels et al. 2019). Reduction in butyrate producing bacteria which are involved in maintaining the barrier integrity is also one of the factors for dysbiosis in IBD (Li et al. 2015). An increase in sulphate reducing bacteria is also seen which produce hydrogen sulphide from sulphate and in turn can block utilization of butyrate and inhibit phagocytosis of bacteria. Thus it is hypothesized that decreased butyrate producing bacteria and increased sulphate reducing bacteria attenuate butyrate production leading to reduced expression of GIT tight junction proteins and increased bacterial translocation. In genetically predisposed subjects, phagocytosis of bacteria is impaired leading to bacterial translocation, toll-like receptor (TLR) activation, stimulation of the immune response and enhanced circulation of inflammatory cytokines (Fava and Danese 2011). Another observation made in IBD is the increase in facultative anaerobes like *E. coli* and reduction of Bifidobacteria. This could be due to disturbance of the anaerobic environment of the intestine as a result of increase in reactive oxygen species facilitating the growth of facultative anaerobes causing dysbiosis (Rigottier-Gois 2013). Bile acids play a major role in lipid absorption, maintenance of cholesterol balance and also act as anti-inflammatory molecules. Gut microbiota are involved in cholesterol assimilation via deconjugation and dehydrogenation of bile acids. Dysbiosis of the gut microbiota is said to impair bile acid metabolism and regulatory inflammatory process. Decrease of Firmicutes and Bacteroides which carry out most of the deconjugation is diminished in IBD leading to escalation of intestinal inflammation (Duboc et al. 2013). Recent studies have also found the role of lipid intake in the aetiology of IBD. A study in Danish individuals reported that excessive consumption of omega-6 polyunsaturated fatty acid (PUFA) enhances the risk of UC by 30% while consumption of omega-3 PUFA lowered the risk of disease by 77% (Tjonneland et al. 2009). Diet plays a major role in the development of several chronic diseases and increases in the levels of omega-6 PUFA in diets such as the Western diet with concurrent reduction in omega-3 PUFA is said to predispose towards a diseased state. Thus, modifying dietary consumption of omega fatty acids would modulate the gut microbiota and in turn modulate dysbiosis and chronic inflammatory conditions. In fact, a study carried out by Kaliannan et al. (2019) reports the importance of balance in the omega-6/omega-3 ratio in causing metabolic endotoxemia. The study reveals an increase in *Enterobacteriaceae* members and decrease of *Bifidobacterium* species in transgenic mice models overproducing omega-6 PUFA. A lower ratio of omega-6/omega-3 on the other hand showed healthy phenotypes. Diet rich in oleic acid (omega-3 PUFA) was found to promote anti-inflammatory gut microbiota and prevent UC in DSS-induced rat model (Fernández et al. 2020).

1.4.2 Obesity

Obesity is a metabolic disorder characterized by excessive storage of body fat in the body as a result of low energy expenditure, high calorie intake and disrupted energy metabolism. However, recent evidences suggest the link of dysbiosis and gut microbiota in the development of obesity (Arslan 2014). There seems to be a characteristic microbiota associated with development of obesity. There is an overall hampered bacterial diversity in the intestine and in most studies it is associated with the ratio of Firmicutes and Bacteroidetes often showing an increase in Firmicutes and decrease in Bacteroidetes (Ley et al. 2005; Turnbaugh et al. 2006). This ratio correlates with body weight and fat accumulation with more obese people having more disproportionate ratio of these phylum species. Studies in germ free mice have found that dysbiosis is most likely to be the causal factor for development of obesity. In a study, both germ free mice and wild-type mice were fed with high fat diet, however only the wild-type mice developed obesity. When microbiota from obese mice were transferred to germ free mice, they became obese (Turnbaugh et al. 2008; Backhed et al. 2007). On the contrary, when lean mice microbiota were transferred to obese mice, the intestinal microbial condition was normalized and alleviated symptoms of metabolic syndrome (Vrieze et al. 2012). These evidences strongly point to the role of gut microbiota in the development of obesity. Diet plays an important role in development of obesity. Consumption of high fat diet is said to lead to a series of changes in the intestine via dysbiosis leading to low grade chronic inflammation. Due to decreased ratio of Firmicutes and Bacteroidetes, there is also a decrease in short-chain fatty acids (SCFAs) as most SCFA producing strains belong to these phyla. SCFAs are said to inhibit fat accumulation in adipose tissue. However, reduction in SCFA production may induce the opposite effect and lead to obese condition (Tilg and Moschen 2014).

In addition to reduced SCFA, dysbiotic microbiota also has effects on the immune system. There is an increase in barrier permeability leading to increased bacterial translocation, triggering production of inflammatory cytokines ultimately leading to chronic inflammation of the intestine and other organs such as liver, adipose tissue, muscles, etc. (Arslan 2014). Lipopolysaccharide (LPS) which is a component of Gram-negative cell wall is said to be the antigen translocating through the intestinal barrier into the systemic circulation when there is an increase in the ratio of Gram-negative pathogens. Translocation of LPS leads to metabolic endotoxemia leading to enhanced chronic inflammation and further to obese state and insulin resistance (Cani et al. 2007).

1.4.3 Diabetes Mellitus

Diabetes mellitus is a metabolic disorder linked to metabolism of carbohydrate characterized by inadequate utilization or production of insulin and consists of Type 1 and Type 2 diabetes. Type 1 diabetes (T1D) also referred to as insulin dependent diabetes mellitus (IDDM) is an autoimmune disease characterized by

deficient insulin production by beta cells in the pancreas and often seen in children or young adults. Type 2 diabetes (T2D) is a condition seen in adults where the tissues become resistant to insulin that is produced and is often referred to non-insulin dependent diabetes mellitus (NIDDM). T2D can be aggravated by obesity and is the most common type of diabetes diagnosed. Although the mechanisms for the development of T1D and T2D are quite different it was found that dysbiosis of gut microbiota is found in both types (Li et al. 2017; Abdellatif and Sarvetnick 2019).

Dysbiosis in T1D is characterized by decrease in Bifidobacteria, *Lactobacillus* and *Prevotella* with a simultaneous increase in Bacteroidetes and *Clostridium* (Wen et al. 2008; McLean et al. 2014). On the other hand, in T2D, there is an increase in *Lactobacillus* and Bacteroidetes with diminished counts of *Clostridium* (Larsen et al. 2010). Nonetheless, both types of diabetes show decreased microbial diversity along with compromised barrier integrity and increased gut permeability (Giongo et al. 2011; Larsen et al. 2010; Qin et al. 2012). Increased bacterial translocation and endotoxemia is also seen in T2D similar to obesity which could lead to low grade inflammation. Prolonged state of this inflammation causes the tissues to develop insulin resistance and sets for the onset of T2D (Cani et al. 2007). Thus dysbiosis of the microbiota is said to trigger the onset of diabetes. It was observed that non-obese diabetic (NOD) mice with T1D had a different microbial composition compared to NOD mice who did not develop T1D (Nielsen et al. 2014). Another observation made is that NOD mice housed in germ free facility developed disease whereas those housed in specific pathogen free (SPF) facility did not (McLean et al. 2014). This is due to reduced expression of myeloid differentiation primary response 88 (MyD88) which is a universal adaptor protein for TLRs. A change in gut microbiota composition and attenuation of diabetes progression was seen in MyD88-/-NOD mice in SPF conditions compared to germ free mice. This report provides additional proof that development of diabetes is dependent on composition of the microbiota in the intestine. MyD88 also plays a crucial role in bacterial recognition and signalling of the innate immune response and knockout of this factor may disrupt the development of IDDM (Duparc et al. 2017).

1.4.4 Colorectal Cancer

Colorectal cancer stands third among the common cancers in the USA and is second leading cause for cancer deaths as stated by the American Cancer Society. The risk factors for colorectal cancer include obesity, diabetes, IBD, diet rich in high fats and proteins which are all linked to intestinal dysbiosis. Thus, development of colorectal cancer is also linked to imbalance in the gut microbial composition (DeGruttola et al. 2016). Colorectal cancer is characterized by decrease in butyrate producing bacteria in addition to increase in several pathogenic bacterial count. Generally, a decrease in Bifidobacteria, *Prevotella* and Proteobacteria correlating with reduced production of SCFA is seen, while there is an increase in counts of Firmicutes, *Enterobacteriaceae*, Bacteroidetes and Fusobacteria specifically *Fusobacterium nucleatum, Peptostreptococcus stomatis* and *Parvimonas micra* (Schulz et al.

2014; Yu et al. 2017). Two specific bacteria which are associated with stimulating strong inflammatory responses, namely *Akkermansia municiphila* and *Fusobacterium nucleatum* were found in high numbers in colorectal cancer (Castellarin et al. 2012). The composition of the intestinal microbiota also differs depending on the severity and stage of cancer. *Enterobacteriaceae* was found to be implicated in patients with polyps while patients with tumours had increased numbers of Bacteroidetes (Sobhani et al. 2011). Experiments have suggested that dysbiosis acts as causal factor for the pathogenesis of colorectal cancer. When germ free C57BL/6 mice received gut microbiota from tumours, it induced tumorigenesis in the colon significantly (Zackular et al. 2013). Antibiotics were also found to show reduction in the growth and number of tumours in colorectal cancer susceptible mice (Zackular et al. 2013). SCFA is said to play a protective role against tumorigenesis evident by the fact that when butyrate was supplemented to high fat diet fed mice, it reduced the incidence of tumours (Schulz et al. 2014). Overproduction of antimicrobial peptide such as α-defensins is said to cause dysbiosis in colorectal cancer patients (Pagnini et al. 2011). Development of chronic inflammation as a result of disturbed microbiota can also lead to colitis-associated colorectal cancer (Elinav et al. 2013).

1.4.5 Irritable Bowel Syndrome

The pathogenesis of irritable bowel syndrome (IBS) is not clearly understood. However, changes in gut microbiota have been observed in IBS patients whose gut microbiota greatly differs from healthy persons (Principi et al. 2018). There is a two fold increase in ratio of Firmicutes to Bacteroidetes in IBS patients than control subjects (Rajilic-Stojanovic et al. 2011; Jeffery et al. 2012). Studies have found increased levels of *Enterobacteriaceae*, *Clostridium* sp. and decrease in Bifidobacteria and *Lactobacillus* spp. (Zhuang et al. 2017). Another study found increase in numbers of Bacteroidetes and *Lactobacillus* spp. while another group observed no differences in Bifidobacteria, *Lactobacillus*, Bacteroides and *Enterococcus* spp. in IBS individuals compared to control subjects (Matto et al. 2005; Ponnusamy et al. 2011). IBS is classified into diarrhoea-predominant and constipation-predominant, each having a characteristic microbiota signature. IBS with predominant diarrhoea showed increase in Proteobacteria and reduced counts of *Lactobacillus*, Bacteroidetes and Actinobacteria (Su et al. 2018; Zhuang et al. 2018). On the other hand, the constipation-predominant IBS patients show increases in Firmicutes count and reduced numbers of lactate-utilizing bacteria such as *Eubacterium hallii* and *Anaerostipes caccae* (Chassard et al. 2012; Gobert et al. 2016). Dysbiosis set inflammation is present in IBS condition demonstrated by the enhanced expression of TLR4 and five responsible for the stimulation of innate immune responses via bacterial recognition pathways (Shukla et al. 2018).

Diet is another crucial component of IBS pathogenesis. Impairment in absorption of dietary carbohydrates causes prolonged production of hydrogen leading to methane build up (Ong et al. 2010). In addition IBS patients have impaired carbohydrate

and protein metabolism linked with changes in the gut bacterial composition (Portincasa et al. 2017). In a cohort study of IBS patients, 52% ascribed their symptoms to dietary constituents. Some related to vegetables (34%), some to fruits (29%), some to fat consumption and milk (15%) while some to peppers, spices and sugar (Tarrerias et al. 2011). A diet low in fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) has been extensively accepted for treatment of IBS although concerns of nutritional inadequacy and safety have been raised (Eswaran et al. 2020). Probiotics and prebiotics have been considered to alleviate the symptoms of IBS or rather restore the gut microbiota. Probiotics have found to modulate the mucosal immune response by improving intestinal barrier integrity and stabilization of gut microbiota (Leventogiannis et al. 2019; Oh et al. 2019b; Li et al. 2020). Symptoms of IBS were found to be alleviated when treated with *Lactobacillus acidophilus* DDS-1 and *Bifidobacterium lactis* UABla-12 (Martoni et al. 2020). Hence, probiotics are being explored as potential strategies for treatment of IBS. However, further studies are needed to validate these claims.

1.4.6 Atherosclerosis

Atherosclerosis is a condition of chronic inflammation of the arteries forming multiple plaques restricting blood flow. Several components of microorganisms or microbial associated molecular patterns (MAMPs) are implicated in atherogenesis (Ahmad et al. 2019). More so several risk factors for development of cardiovascular disease and the metabolism for phosphatidylcholine are dependent on composition of gut microbiota (Wang et al. 2011). Certain bacterial species are found associated with plaques in oral and gut samples of atherosclerosis patients (Wu et al. 2011). Faecal sample analysis of atherosclerotic patients showed a dominance of *Ruminococcus* and reduced expression of Bacteroides enterotypes. The microbiome of the patients harboured a rich pool of genes coding for peptidoglycan synthesis, but poor in genes coding for phytoene dehydrogenase involved in metabolism of lipid-soluble antioxidants (Karlsson et al. 2012). Altered Firmicutes-Bacteroidetes ratio could also lead to development of cardiovascular diseases (CVD) (Callejo et al. 2018). Gut microbiota metabolites such as SCFA, bile acids and trimethylamine-N-oxide (TMAO) are correlated to pathogenesis of CVD (Tang et al. 2017; Kitai and Tang 2018). Studies on link of gut microbiota and atherosclerosis are limited but present a strong evidence for potential therapeutic strategies by targeting gut microbiota for the prevention and treatment of atherosclerosis.

1.4.7 NAFLD

Non-alcoholic fatty liver disease (NAFLD) is a collective term for conditions of the liver in people who consume little or no alcohol. The risk factors or causes of NAFLD include obesity, high cholesterol, T2D, metabolic syndrome, hypothyroidism, polycystic ovary syndrome, etc. Advanced stages of NAFLD include

inflammation of liver leading to cirrhosis and liver failure. Overgrowth of small intestinal bacteria along with high concentrations of products such as trimethylamine, TMAO, acetaldehyde and tumour necrosis factor (TNF)-α is linked to aetiology of NAFLD (Kaur et al. 2020). Since the liver is well connected to the GIT, it is easily affected by bacterial translocation, endotoxins, inflammatory cytokines, etc. Microbiota associated with obese phenotype are found to induce hepatic triglyceride synthesis and lipid metabolism directly affecting storage of fats in liver (Backhed et al. 2004). Thus, dysbiosis or bacterial overgrowth causes intestinal permeability and hepatic steatosis in obese subjects (Sabate et al. 2008). Chronic NAFLD is also associated with high endotoxin infiltration (Verdam et al. 2011). Increase in γ–Proteobacteria and *Erysipelotrichia* is said to be linked to development of fatty liver (Spencer et al. 2011). Changes in numbers of Proteobacteria, *Enterobacteriaceae*, *Lachnospiraceae*, *Escherichia* and *Bacteroides* are commonly observed in NAFLD (Zhu et al. 2013; Zhao et al. 2019). Disparity in the ratio of Bacteroides and Firmicutes was observed in a study conducted by Zhu et al. (2013) in faecal samples of obese subjects and non-alcoholic steatohepatitis (NASH) children. Thus, changes in bacterial phyla could help predict risks for the development of fatty liver.

1.4.8 Coeliac Disease

Coeliac disease is an autoimmune disease characterized by heightened immune response to certain peptides found in gluten and is said to be linked to changes in gut microbiota. Presence of genes HLA-DR3 and HLA-DQ2 increases risk of development of coeliac disease (1). A study conducted by De Palma et al. (2010) found that HLA-DQ genotype influences the colonization of the gut favouring Gram-negative bacteria especially from the *Bacteroides-Prevotella* group. A reduced proportion of *Bifidobacterium* was also observed in the same genotype. In addition, peptides derived from gliadin during digestion induce the secretion of pro-inflammatory cytokines directly impacting pathogenesis of coeliac disease (Girbovan et al. 2017). Dysbiotic microbiota of coeliac disease patients alter the expression of TLR-2, TLR-9 further maintaining the dysbiotic condition (Kalliomäki et al. 2012). In coeliac disease, there is a specific Th1 and Th17 immune response against certain gluten peptides (Jabri and Sollid 2009). SCFA concentrations are found to be altered in coeliac affected patients which points to a possible role of microbiota in modulating oral tolerance. Thus, dysbiosis could be a risk factor by directly inducing mucosal inflammation or by pro-inflammatory response to gluten. Although Gram-negative bacteria are main indicators of a dysbiotic microbiota in coeliac disease patients, pathogenic Gram-positive bacteria such as *Staphylococcus, Clostridium* and *Actinomyces* spp. were isolated from affected patients (Nistal et al. 2012; Bodkhe et al. 2019). Supplementation of probiotics and gluten free-diet are envisaged as therapeutic approaches for management of coeliac disease (Chibbar and Dieleman 2019).

1.4.9 Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by inflamed joints. Intestinal commensal bacteria were found to be involved in the development and progression of disease. In a cohort study of RA patients, *Prevotella copri* was found to correlate with the onset of disease (Scher et al. 2013; Maeda et al. 2016; Kishikawa et al. 2020). Recently, *Lactobacillus salivarius* was demonstrated to be a marker for the development of RA through a metagenome-wide association study (Zhang et al. 2015). Surprisingly, many of these studies found transmission of oral species from the mouth to the gut in RA patients (Schmidt et al. 2019). Metabolomics analysis of diseased patients showed several altered metabolic functions including redox mechanism, metal ion metabolism and arginine metabolism (Zhang et al. 2015). Studies on link of intestinal bacteria to RA are still limited and require future studies in this regard.

1.4.10 Asthma

Colonization and development of the intestinal microbiota in initial years of life are crucial in determining the health of an individual or susceptibility to disease in later stages of life. Exposure to antibiotics and germ free conditions in infancy is said to increase the risk of developing conditions such as asthma and allergy (Risnes et al. 2011). Early colonization of bacteria also helps in shaping and tutoring the immune system in the absence of which levels of IgE, basophil and T-regulatory cells are high. Thus it is postulated that microbiota regulate the levels of IgE and basophils via B cell-intrinsic MyD88 signalling. In absence of intestinal microbiota B cells preferentially shift to isotype IgE instead of IgA leading to allergic inflammatory responses (Cahenzli et al. 2013). The lung which was previously considered sterile is found to harbour microbiota majorly belonging to phyla Actinobacteria, Firmicutes, and Bacteroidetes. In clinical studies, dysbiosis of lung microbiota with an abundance of Proteobacteria genera such as *Haemophilus* and *Moraxella* was said to be associated with development of asthma (Teo et al. 2018; McCauley et al. 2019; Hufnagl et al. 2020). Infants showing risk factor for asthma development showed changes in gut microbial composition along with low levels of faecal acetate. Asthmatic children projected significantly low abundance of *Faecalibacterium* and *Roseburia,* whereas proportions of *Clostridium* and *Enterococcus* were higher compared to healthy controls (Arrieta et al. 2015; Stiemsma et al. 2016). Increasing the counts of four bacterial genera found in low levels in asthma risk children alleviated inflammation of the airway in germ free mice (Arrieta et al. 2015). Invariant natural killer T cells (iNKT) are also found to be implicated in asthma. Germ free mice which showed signs of asthma development had high levels of iNKT cells in their lungs and intestine.

1.4.11 Multiple Sclerosis

Multiple sclerosis (MS) is a disease of the central nervous system mediated by the immune system. Several factors including genetic and environmental are responsible for the pathogenesis of this disease. A reduced microbial diversity is observed in patients with multiple sclerosis compared to healthy individuals (Chen et al. 2016). This includes reduction in species of *Faecalibacterium prausnitzii*, *Eubacterium rectale* and SCFA producing bacteria while there was significant increase in numbers of *Streptococcus thermophilus/salivarius*, *Eggerthella lenta* and *Prevotella* sp. (Atarashi et al. 2013; Jangi et al. 2016; Zeng et al. 2019). Like RA, several studies have indicated an increase in the species of *Prevotella* in MS individuals although the exact cause of this is not established (Miyake et al. 2015; Jangi et al. 2016; Zeng et al. 2019). Through animal models of experimental autoimmune encephalomyelitis (EAE), it is established that microbial colonization is necessary for development of EAE via activation of $CD4^+$ T cells while the germ free mice did not show any disease symptoms (Berer et al. 2011). Further investigations into the contributions of gut microorganisms especially SCFA producing strains in pathogenesis of MS are warranted.

1.4.12 Aging

The composition of the gut microbiota is affected as age progresses. There is an increase in the number of Gram-negative bacteria which secrete LPS and in turn induce inflammatory tone in the gut (Kumar et al. 2016). Dysbiosis also includes reduction in butyrate producers, Bacteroidetes population and Firmicutes/Bacteroides ratio reported by 16S Sanger sequencing and next-generation pyrosequencing (Picca et al. 2018a). Several signalling pathways including inflammation, oncogenesis and brain function related to anxiety are also said to be affected by gut microbiota (Murphy et al. 2014). SCFAs which play a protective and anti-inflammatory role in the gut are also reduced in the elderly and may exacerbate the immune system (Choi et al. 2018). Several reports have demonstrated the bidirectional link between the gut microbiota and the brain termed as the 'gut-brain axis'. The gut microbiota not only influences the development of the central nervous system but also affects neurological behaviour such as anxiety and depression (O'Mahony et al. 2014). Aging is a health concern around the world since it deals not only with loss of gut microbiome diversity and physiological function but also a reduction in physical power such as muscle power and digestive ability (Thevaranjan et al. 2017).

Alzheimer's disease (AD) and Parkinson's disease (PD) are both neurodegenerative diseases associated with aging. A correlation was established between gut microbiota and AD. Studies have found that a neurotrophin, namely brain-derived neurotrophic factor (BDNF) associated with cognitive function and synaptic plasticity is decreased in germ free animals and AD patients (Huang and Reichardt 2001; Leung and Thuret 2015). However, probiotic administration restored the expression

of BDNF and improved memory, learning and cognitive function (Abraham et al. 2019; Bonfili et al. 2020). PD is commonly associated with gastrointestinal problems (Hill-Burns et al. 2017). Increased intestinal permeability and inflammation as a result of gut dysbiosis could lead to abnormal gut-brain axis, misfolding of α-synuclein, inflammation of the brain and dopaminergic neuron damage, all of which are implicated in the pathogenesis of PD (Choi et al. 2018).

Sarcopenia is an age-related condition characterized by loss of muscle mass and strength (muscle wasting) leading to physical disability and poor quality of life. The link between the gut microbiota and muscle wasting during aging is believed to be associated with amino acid bioavailability regulation. Dysbiosis of the gut microbiota impacts the bioavailability of amino acids as they are obtained by the hydrolysis of proteins from microbiota-derived proteases and peptidases (Rowland et al. 2018). Additionally, chronic low grade inflammation ('inflammaging') along with increased intestinal permeability aggravates dysbiosis and development of muscle wasting during aging via gut microbiota-muscle crosstalk. Increased activities of AMP-activated protein kinase (AMPK), carnitine: palmitoyltransferase-1 (CPT-1) and fasting-induced adipocyte factors tend to counteract the effects of fasting and denervation on muscle wasting. Mitochondrial dysfunction and systemic inflammation play a critical role in sarcopenia (Picca et al. 2018b). In fact, studies have reported that mitochondrial DNA which act as damage associated molecular pattern (DAMP) and are extruded from damaged mitochondria. This DAMP activates the innate immune response and induces the production of pro-inflammatory molecules cascading a vicious cycle of increased mitochondrial damage, inflammation, production of reactive oxygen species, etc. in the myocytes eventually causing muscle wasting (Picca et al. 2018a). A significant decrease in the numbers of lactobacilli, *Faecalibacterium prausnitzii* and *Bacteroides/Prevotella* together with an increase in proportions of *Enterobacteriaceae, Atopobium* and *Ruminococcus* is seen in elderly with high frailty scores (van Tongeren et al. 2005; Ticinesi et al. 2017). Metagenomics analysis of aged subjects called the ELDERMET study clearly indicated the role of butyrate producing bacteria with healthy aging function (Claesson et al. 2012). Hence, prebiotic and probiotic supplementation to boost butyrate producers has been suggested for age-related muscle dysfunctions.

Interestingly, connection between the gut microbiota and heart disease is increasingly being reported. The gut microbiota in patients with heart failure seems to be altered with an increase in the quantities of pathogenic bacteria, enhanced intestinal permeability and systemic inflammation, classical mechanisms as seen in the above-mentioned conditions (Kamo et al. 2017). Gut microbiota-derived metabolites such as TMAO and indoxyl sulphate could possibly contribute to heart failure pathogenesis by mechanisms yet to be understood (Tang et al. 2014; Organ et al. 2016). The newer concept of heart-gut axis could open new avenues for understanding the pathophysiology of cardiovascular diseases and finding potential treatments.

1.4.13 Adverse Pregnancy Outcomes

Pregnancy is a complex physiological process accompanied by significant hormonal, metabolic and immune changes. These changes have an obvious impact on the microbiota of not only the gut but also changes in vaginal, oral and placental microbiota. However, unlike other conditions, these changes are necessary and facilitate healthy pregnancy duration both for the mother and the foetus. During pregnancy, there is a dramatic increase in oestrogen and progesterone levels which is likely to affect the gut microbe composition. Additionally, microbiota are also known to secrete certain hormones indicative of a bidirectional interplay between gut microbiota and hormones. Changes in the immune system include protection of mother-child from infections, development of foetal immune system and preventing foetal rejection by the mother's immune system. These changes could affect the microbiota composition or vice versa. Metabolic changes include weight gain, gestational diabetes mellitus (GDM), low grade inflammation again actively associated with microbiota changes (Neuman and Koren 2017). An increase in proportions of Actinobacteria and Proteobacteria and decreased abundance of *Faecalibacterium* is observed similar to metabolic syndrome (Haro et al. 2016). Gut microbiota contribute to weight gain of host by increased nutrient absorption, increased secretion of fasting-induced adipocyte factor secretion, immune modulation and induction of catabolic pathways (Koren et al. 2012).

Diet, health of the host, antibiotics also play a vital role in the gut microbiota composition during pregnancy. GDM and obesity are serious concerns since they pose threat to adverse pregnancy outcomes. Maternal obesity is related to adverse pregnancy outcomes such as GDM, gestational hypertension, preeclampsia, preterm birth, foetal defects, caesarean delivery and perinatal death (Marchi et al. 2015). GDM poses risk of spontaneous abortion, foetal defects, preeclampsia, macrosomia, neonatal hypocalcaemia, neonatal hypoglycaemia, neonatal hyperbilirubinemia and neonatal respiratory problems (Spaight et al. 2016). An enrichment of *Parabacteroides distasonis* and *Klebsiella variicola* was found in second trimester pregnant women with GDM (Kuang et al. 2017). On the other hand, reduced *Bacteroides* and *Bifidobacterium* and increased *Escherichia coli* and *Enterobacteriaceae* are seen in overweight pregnant women (Santacruz et al. 2010). Bacterial infections have been seen in pregnancy complications the mechanisms of which are yet to be elucidated (Fox and Eichelberger 2015). Few studies demonstrated a correlation between preterm birth and α diversity in gut microbiota (Stout et al. 2013). Reports have also implicated dysbiosis of vaginal, oral and placental microbiome to adverse pregnancy outcomes. Increased abundance of *Gardnerella* and *Ureaplasma*, α diversity and lower abundance of *Lactobacillus* sp. as well as increased numbers of *Candida albicans* in vagina was found implicated in preterm birth (Romero et al. 2014). *Fusobacterium nucleatum*, a non-pathogenic oral anaerobe has been found to haematogenously spread to the placenta and increase endothelium permeability further allowing colonization of pathogenic organisms (Fardini et al. 2011). Such studies suggest the possible link of periodontal disease to preterm birth.

1.5 Diet and Microbiome Interplay

After birth, the colonization of the infant GIT is largely determined by diet which acts as one of the major factors which drives the development and maintenance of the gut microbiota. This is evident by the fact that the gut microbial composition of breast fed infants differs from their formula fed counterparts (Albenberg and Wu 2014). The effect of specific type of foods and components of diet on human body has been investigated over a period of years and has found that diet profoundly influences the composition of the gut microbiome and plays a role in causing dysbiosis correlating with a host of diseases such as metabolic syndrome, obesity, diabetes, cancer, IBD, etc. (DeGruttola et al. 2016).

The so-called Western diet is rich in sugar, animal fat and protein while an agrarian diet is composed of simple sugars, carbohydrates but low in saturated animal fat and protein. The gut microbiota of people consuming Western diet showed a high incidence of Firmicutes and Proteobacteria and few Bacteroides while the agrarian diet showed dominance of Actinobacteria mostly belonging to *Prevotella* species (De Filippo et al. 2010). This implies that diet can skew the balance of microorganisms in the gut favouring particular groups depending on diet intake. Increase of groups that are pathogenic or those which can elicit an immune response along with depletion of the beneficial species leads to dysbiotic state of the gut. Disrupted barrier permeability is one of the factors correlated with dysbiosis when mice were fed a high fat and high sugar diet (Li et al. 2015). SCFAs are metabolic products of fermentation of fibre by commensal bacteria and SCFA such as butyrate are known to play a protective role against pathogenic microbes. Thus diet rich in fibre may have a regulatory effect in incidence of dysbiosis and may help prevent diseases such as colorectal cancer and IBD (DeGruttola et al. 2016).

Vitamin D has shown some positive effects in reduction of IBD and colitis risk. Vitamin D was found to reduce the frequency of diarrhoea and improve body weight in colitis model of mice induced with dextran sulphate sodium (Lee et al. 2015). Vitamin D is also said to have an anti-inflammatory effect and improves resistance to injury while also speculated to regulate the gut microbial homeostasis. Mice with vitamin D receptor deficiency have shown an increase in the levels of *Clostridium* and Bacteroidetes with a reduction in Firmicutes and lactobacilli count (Jin et al. 2015; Assa et al. 2015). Deficiency of vitamin D and lactobacilli could lead to potential dysbiosis and increase the risk of chronic inflammation leading to the development of colitis and further colorectal cancer. Lactobacilli are known to be one of the potential probiotic bacteria which inhibit pathogenic microbes by production of lactic acid and have shown anti-inflammatory and anti-carcinogenic activity (Jin et al. 2015; Archer et al. 2018). In an experimental study in vitamin D deficient mice, intestinal barrier function was found to be disrupted leading to enhanced translocation of bacteria, chronic low grade inflammation and in turn increasing the risk of inflammatory diseases (Ooi et al. 2013; Assa et al. 2015). This implies that vitamin D also helps in maintenance of gut barrier function.

Consumption of whole grains and dietary fibre is strongly associated with reduction in risk of colorectal cancer, diabetes, obesity, CVD, etc. Whole grains

are said to be rich in phenolics such as ferulic acid which are converted to dihydroferulic acid and thought to have a protective effect against neoplastic differentiation of colonic epithelial cells. In addition, ferulic acid via consumption of whole grains and its break down product has shown to boost immune response, possess antimicrobial property and decrease the risk of obesity. Intake of whole grains is also said to positively influence the levels of Bacteroides and lactobacilli and also reduce TNF-α (Vitaglione et al. 2015). On the contrary, reduced intake of fibre is associated with increased risk of advanced colorectal cancer (Oh et al. 2019a). Patients suffering from advanced colorectal cancer were found to possess very low levels of butyrate producing bacteria. Increased intake of dietary fibre is shown to improve their count and reduce the risk of colorectal cancer (Chen et al. 2013). Intake of dietary fibre and whole grains is said to selectively promote the growth of butyrate producing Bifidobacteria and *Roseburia*. A high protein-low carbohydrate diet versus a high protein-moderate carbohydrate diet study showed that dietary fibre contributed to preventing risk of colorectal cancer development via promoting production of SCFA producing bacteria, modulation of immune response and also preventing the formation of carcinogenic N-nitroso compound (NOC) formed during increased intake of meat (Russell et al. 2011).

Fruits and vegetables are high in dietary fibre content and have a chemo protective effect in colorectal cancer (Kunzmann et al. 2016). Consumption of red wine (rich in polyphenols) has also shown to ameliorate dysbiosis in humans stimulating the concentrations of probiotic bacteria such as Bifidobacteria, *Prevotella* and Proteobacteria while inhibiting pathogens like *Clostridium* (Queipo-Ortuño et al. 2012). Polyphenols have a beneficial effect on the gut microbiota and have antimicrobial functions and can be found in foods such as wine, fruits, tea, chocolate and vegetables. Polyphenols are also widely known to lower the risk of cardiovascular disease by reducing hypertension and blood cholesterol levels (Mendonça et al. 2019). Indigenous fermented foods which have been consumed since ages have recently received wide attention for their health promoting effects as they are rich in vitamins, minerals, polyphenols and probiotic microorganisms and their products such as lactic acid (Archer and Halami 2017).

In a humanized mouse model study, faecal microbiota from an adult human was transplanted to a germ free mice and after a shift of diet to Western diet from initial low fat, plant carbohydrate rich diet, the gut microbiota composition showed an increase in counts of Firmicutes including *Clostridium, Enterococcus* spp. and subsequent reduction in Bacteroides (Turnbaugh et al. 2009). Complex carbohydrates are shown to promote the growth of beneficial flora such as Bifidobacteria and may negate the counts of *Enterobacteriaceae* group and *Mycobacterium avium* subsp. *paratuberculosis* (Walker et al. 2011). Refined sugars appear to stimulate the growth of *Clostridium difficile* and *Clostridium perfringens* via increased bile output (Begley et al. 2006). Alterations in the gut microbial composition affect the metabolic and inflammatory pathways of the host. Protein rich diets are said to enhance activity of bacterial enzymes such as azoreductase, nitroreductase and β-glucuronidase. Presence of some members of the microbiota such as Firmicutes, Actinobacteria, Bacteroidetes, etc. is beneficial and is capable of

degrading complex carbohydrates as well as inhibits growth of pathogens such as *Clostridium* and pathogenic *E.coli* (Flint et al. 2012; Parnell and Reimer 2012).

1.6 Strategies to Alleviate Dysbiosis

Advances in techniques and technologies have generated vast knowledge in the field of human microbiome and cross-talk between the microbiota and host. This repository of knowledge has also made possible to develop strategies to intervene dysbiotic states leading to disease. Conventional strategies include changes in diet as described above, antibiotics or even direct or indirect administration of exogenous microorganisms such as probiotics, faecal microbial transplantation and prebiotics. These interventions may alleviate dysbiosis by production of antimicrobial proteins, stimulating the immune response, improving the barrier function, etc. Probiotics are beneficial microorganisms when consumed in sufficient quantities are known to affect several health benefits on the host. However, the effects of probiotics are strain specific and are required to be consumed over a long period of time for colonization of the host GIT. Some are capable of establishing in the GIT while some are processed by the natural flushing mechanism of the gut (Plaza-Diaz et al. 2019). Certain probiotic products such as fermented milk are shown to increase weight in severe acute malnutrition by alleviating dysbiosis while certain strains of probiotics have shown anti-obesity effects (Koutnikova et al. 2019; Chileshe et al. 2020). Administration of specific strains of probiotics in clinical trials in children has demonstrated attenuation of several pathologies such as IBD, colic, allergies, nosocomial and antibiotic-associated diarrhoea, necrotizing enterocolitis (NEC) (AlFaleh and Anabrees 2014; Chau et al. 2015; Korpela et al. 2016; Zhang et al. 2016; Dhama et al. 2016). In adults, several clinical studies have proven effects of probiotic strains against dysbiotic conditions such as obesity, diabetes, aging related conditions, gastrointestinal conditions, allergies, adverse pregnancy related outcomes (Ballini et al. 2019; Cancello et al. 2019; Madempudi et al. 2019; Marißen et al. 2019; Tamtaji et al. 2019; Ahmadi et al. 2020; Lombardi et al. 2020). Furthermore, studies are conducted to isolate indigenous probiotic bacteria and develop strain specific probiotics/consortium with specific health benefits. Prebiotics are substances that selectively promote the growth of microbes conferring a health benefit to the host (Gibson et al. 2017). The concept of prebiotics is expanded from typically consisting of non-digestible carbohydrates to a broad array of compounds. Prebiotics are thought to have a pivotal relationship with gut microbiota. In fact human milk oligosaccharides (HMOs) nourish the gut microbiota of developing infants. One of the most common effect of prebiotics is the increase in the count of *Bifidobacterium* and *Lactobacillus* sp. which contribute to several health beneficial functions in the host such as reduction of inflammation. Promotion of growth of beneficial microorganisms also facilitates the release of SCFAs which display anti-cancerous properties as well as promote the absorption of minerals in the host gut. Galactooligosaccharides (GOSs) were found to increase the abundance of *Lactobacillus*, *Faecalibacterium* and *Bifidobacterium* in humans with dietary intolerance

(Azcarate-Peril et al. 2017). Another important mechanism of prebiotics was demonstrated using short-chain fructooligosaccharide (FOS) and inulin which showed improved ability in maintenance of epithelial barrier function and prevention of injury from non-invasive enterohaemorrhagic pathogen *E.coli* 0157:H7 (EHEC) (Wu et al. 2017). Deciphering the complex nature and prebiotic utilization of the gut microbial species could help investigate the effects of a broad range of prebiotics in our diet on the gut microbiome composition and dysbiosis (Altamura et al. 2020). Faecal microbiota transplantation (FMT) is a method of bacteriotherapy which involves transplantation of faeces from healthy individuals to patients with dysbiosis (Bauer et al. 2020). Earlier FMT was done through faecal enemas, colonoscopic administration and through nasoduodenal tubes. However, non-invasive frozen inoculum capsules have been developed which can be taken orally with no reported side effects (Youngster et al. 2014). The effects of FMT have been demonstrated in *Clostridium difficile* infection showing about 95% remission globally (Aroniadis and Brandt 2013). FMT is being explored for other diseases such as IBD and insulin resistance (Vrieze et al. 2012; Ianiro et al. 2014). FMT in patients with IBD showed a shift towards phyla characteristic of the donor such as *Faecalibacterium prausnitzii, Roseburia faecis* and *Bacteroides ovatus* (Ianiro et al. 2014). However, more intense studies are required to determine the efficiency of FMT in IBD. Ethical issues, cost, safety and health risks are some of the other concerns associated with FMT.

1.7 Conclusion

Dysbiosis is a complex condition associated with the gut microbiota and increasingly believed to play a role in the pathogenesis of several diseases. Recently gut microbiota is being linked to mental health and found to predispose towards neurological disorders. Factors such as diet, genetics and environmental factors need to be investigated in detail which have an impact on the development of gut microbiota and its changes. Future studies on characterization of gut microbial diversity in healthy and specific diseased individuals and identifying mechanisms involved are required to develop interventions for dysbiosis and diseases correlated to it.

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Probiotic Based Interventions for Improving Intestinal Health

2

Kezia Devarapalli, Praveena Ganji, Chandrakala Gunturu, Prakasham Reddy Shetty, and Linga Banoth

Abstract

Probiotics are beneficial microbial strains used for improving intestinal health, through the modulation of the microbiota of the gut. These microbes play a critical role in regulating nutrition, metabolism, physiology, and immunity of human. Gut microbes are unique in nature and are non-pathogenic with anti-inflammatory potential and are responsible for maintenance of resistance in the intestine. This paper provides a broad review on promising intestinal probiotic properties of *Lactobacillus* sp., *Bifidobacterium* sp., and other yeast species, and their role in the prevention of acute gastroenteritis, inflammatory bowel disease, atopic dermatitis, allergic asthma, food allergy, food-induced anaphylaxis, *Candida* infection, colic infection, enter colitis, *H. pylori* infection, celiac disease, ulcerative colitis along with the importance of probiotic-rich foods for gut health.

Keywords

Intestinal health · Microbiota · Gastrointestinal diseases · *H. Pylori* infection · Probiotic-rich foods for gut health

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Abbreviations

CD	Celiac disease
DOB	Delta over baseline
EFFCA	European food and feed cultures association
EFSA	European food safety authority
GI	Gastrointestinal
IBD	Inflammatory bowel disease
IKB	I Kappa B protein
LAB	Lactic acid bacteria
MALT	Mucosa-associated lymphoid tissue lymphoma
NF-kB	Nuclear factor kappa B
QPS	Qualified presumption of safety
SCFAs	Short chain fatty acids
UC	Ulcerative colitis

2.1 Introduction

The health benefits to humankind such as improved health and delay of senility are mainly associated with intestinal microbiota, which is regularly supplemented by yogurt and/or fermented food(s) which have been well documented in the literature before microbes were ascertained. However the idea of administering healthy microbes to confer a positive impact was noticed several decades ago. The word "probiotic" was first coined in (1954) for a group of microbes or microbial species which confer a health benefit to the host. According to Elie Metchnikoff in his terms *"The dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes" and "systematic investigations should be made on the relation of gut microbes to precocious old age, and on the influence of diets which prevent intestinal putrefaction in prolonging life and maintaining the forces of the body."* Henry Tissier in (1906) also noticed the young children suffering with diarrhea, that Y-shaped ("bifid") microbial strains present in stool of healthy pupils play a significant curative role and restore a healthy gut microbiota. Realization of the potential helpful bacterial population by researchers and health workers along with advanced molecular biology techniques led to increased inquest for exploring the basic involvement of probiotic microbial role in modulating the gut environment towards health by researchers and health workers in spite of the complexity of the gut ecosystem. Considering the probiotic microbes versus human health, regulatory bodies around the world such as the World Health Organization (WHO), Food and Agriculture Organization of United Nations (FAO), International Scientific Association for Probiotics and Prebiotics (ISAPP), European Food Safety Authority (EFSA), and the U.S. Food and Drug Administration (FDA) swung in defining the term probiotics like live microbes, when taken in sufficient amounts, confer a health

benefit on the host. However, the conflict is continuing in understanding the term "health claim quantum" with respect to the verity that market players have outperformed the aptitude of science inputs to substantiate the evidence (Breidt et al. 2013). However, major health claims, including the use of probiotics in the treatment and cure of disease, are quotable and can be effectively proven with research activities similar to studies conducted for drugs.

2.2 Probiotics Market Size, Share, and Trend Analysis

The global probiotics market size was estimated at USD 48.38 billion in (2018) and is anticipated to expand at a compound annual growth rate (*CAGR*) of 6.9% during the forecast period https://www.researchandmarkets.com/r/93d2r3 and mainly driven by the growing consumer inclination towards preventive healthcare in conjunction with development of efficient probiotic strains.

Regulations pertaining to the use of probiotics in nutraceuticals, nutricosmetics, and dietary supplements, especially infant formulas are extremely stringent in the USA and are expected to pose a challenge for the regional market players. This has resulted in stagnancy in innovation in probiotic products for baby boomers. On the other hand, in 2016, 204 probiotics-based products for adults were launched in the country.

2.3 What Are Probiotics?

Ever since the realization of probiotics microbial role in the health sector, various efforts have been made rapidly to evaluate each and every microbe involvement in providing positive health benefit to humankind. Probiotics include specific live bacterial or yeast strains mainly associated with fermented foods like sauerkraut, miso, tempeh, kimchi, and kefir. Up on consumption, they accumulate/habitat in the gut and improve their ratio against bad bacteria which play vital role in improving the health. Imbalance of probiotic bacterial ratio leads to autoimmune related problems (like thyroid issues, rheumatoid arthritis, type 1 diabetes, etc.) in addition to digestive issues (irritable bowel syndrome, constipation, diarrhea, heartburn, or bloating). Examples of some good and bad bacteria are shown in (Fig. 2.1)

2.4 Health Improvement by Using Probiotics

Probiotics mainly function to create a balance in a healthy digestive system. The imbalance between good and the horrific microbial population, either may be due to illness or treatment system induced medicated effect such as antibiotic administration, insufficient nutrient diet, or the out raising (Chyn et al. 2019) of unfriendly bacteria or any other gastrointestinal problems. With the existing literature, there are evidences that probiotics may facilitate in:

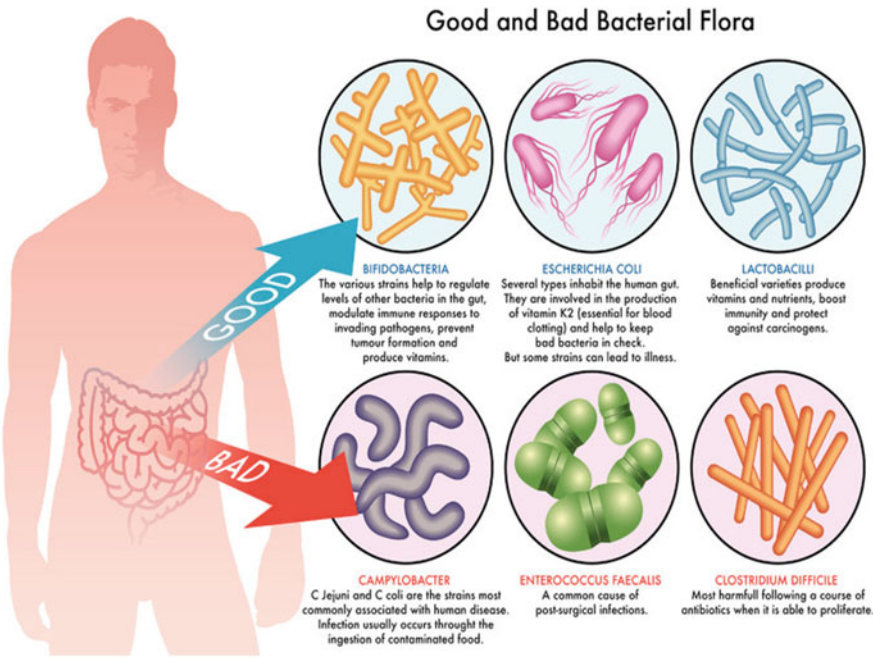

Fig. 2.1 Good and bad bacterial Flora. Source: https://www.canstockphoto.com/support.php

- Prevention and/or treating diarrhea caused by infectious microbes and antibiotics
- Improving symptoms associated with irritable bowel syndrome
- Boosting of the immune system
- Reduction of inflammation and allergies

In general, probiotic microbes belong mainly to genus *Lactobacillus* and *Bifidobacteria* with a slight variation in the functioning of probiotic characteristics. In addition, a sporadic distribution of probiotic microbes in bacterial as well as yeast genera too, such as *Bacillus, Escherichia*, *Streptococcus*, *Enterococcus, Propionibacterium, Leuconostoc, and Saccharomyces* for classical example, please see (Table.2.1).

2.5 Probiotic Properties of Major Intestinal Bacteria

2.5.1 Probiotic Properties of Specific Strains in Genus *Lactobacillus*

Eighty four *Lactobacillus* species certified by the European food and feed cultures association (EFFCA) as safe human consumption and are effective for technological and beneficial use (Baohong et al. 2017). According to European Food Safety Authority (EFSA) another 36 species of genus Lactobacilli have the status of Qualified Presumption of Safety (QPS) (Kennedy 1995). *In general, the probiotic*

Table 2.1 Health benefits of commercially available probiotic microorganisms

Strain	Commercial product/ available form	Source	Health benefits	Reference
Lactobacillus rhamnosus GG	Culturelle; Dannon Danimals	Valio Dairy (Helsinki, Finland)	Protects intestinal barrier functions like altered goblet cells, the mucus layer counteracts variations in lymphocytes, increases the genes expression related to gut permeability: motility, absorption, cell proliferation, and protective functions through endogenous proteases inhibition *L. Rhamnosus* CNCMI- 3690; Counter pathogenic bacteria and fungi in the urogenital tract- *L. rhamnosus* GR-1. Improves intestinal permeability and modulates microbiota dysbiosis – *L. rhamnosus* GG (LGG)	(Hong et al. 2013; Koji et al. 2017; Kuipers 1997)
Lactobacillus acidophilus NCFM	Sold as ingredient	Danisco (Madison, WI)	Ulcerative colitis ; *L. acidophilus* antagonistic function is similar to *Staphylococcus aureus, Escherichia coli, Salmonella typhimurium,* and *Clostridium perfringens* ; Treatment of pediatric diarrhea	(Cho et al. 2006; Christoph et al. 2017; Gayathri and Rashmi 2016)
Lactobacillus plantarum OM	Sold as ingredient	Bio-Energy Systems, Inc. (Kalispell, MT)	Treatment of various diseases like inflammatory bowel disease, diarrhea, dermatitis (atopic), obesity, insulin resistance syndrome, type 2 diabetes, and nonalcoholic fatty liver disease; Cure for	(Chyn et al. 2019; Gijsbers et al. 2016; Ishikawa 2003)

(continued)

Table 2.1 (continued)

Strain	Commercial product/ available form	Source	Health benefits	Reference
			autoimmune disorders and inflammation- *Lactobacillus plantarum* 299V	
Lactobacillus casei DN-114001	Dan Active fermented milk	Danone (Paris, France)	Treatment of colitis-associated colorectal cancer -*Lactobacillus casei* BL23; Protects against nonalcoholic steatohepatitis; eases abdominal dysfunction in normal medical students exposed to academic Stress- *Lactobacillus casei* strain Shirota (LcS)	(Akito et al. 2016; Chen et al. 2007; Elsa et al. 2017)
L. delbrueckii subsp. *bulgaricus*	Grainfields wholegrain liquid	AGM Foods Pvt. Ltd., Australia	Decreases triglycerides, LDL levels, total cholesterol; increases immunity and fight viruses as well as leaky gut symptoms, diarrhea & nausea, inflammation and tooth decay; Improves dairy digestion and IBS symptoms; manages HIV symptoms; fights dyspepsia	(Cevikbas et al. 1994; Galdeano and Perdigón 2007; Gómez et al. 2017)
L. brevis	–	–	Prevents dental caries and also tooth decay, -*Lactobacillus brevis* BBE-Y52 helps through antimicrobial activity; *Lactobacillus brevis* KB 290 helps in treating influenza	(Denis et al. 2005; Moraes et al. 2014)
L. johnsonii	–	–	Mainly helps in immunomodulation and reduces gastritis as well as *H. pylori* related symptoms	(Franqois et al. 1991; Fridman et al. 2017; Guslandi et al. 2000)

(continued)

Table 2.1 (continued)

Strain	Commercial product/ available form	Source	Health benefits	Reference
Lactobacillus fermentum VRI003 (PCC)	Sold as ingredient	Probiomics (Eveleigh, Australia)	Helps in cholesterol assimilation; *L. fermentum* AGR1487 helps in maintaining intestinal barrier integrity; *L. fermentum* ME-3 mediated antimicrobial and antioxidative activities	(Imase et al. 2007; Kang et al. 2016; Messina1995)
Lactobacillus reuteri ATCC 55730	BioGaia probiotic chewable tablets or drops	Biogaia (Stockholm, Sweden)	Function as antimicrobial and immunomodulatory agent by modulating host microbiota also has role in neuromodulatory capability	(D'arienzo et al. 2011; Dylag et al. 2014: Elsa et al. 2017; Najma et al. 2016)
Bifidobacterium infantis 35264	Align	Procter and Gamble (Mason, OH)	Treatment of irritable bowel syndrome, Ulcerative colitis (UC) - *B. infantis*3562; Increases growth of very-low-birth weight infants (VLBWI)	(Aagaard et al. 2012; Jamie et al. 2015)
Bifidobacterium lactis Bb-12	Sold as ingredient	Danisco (Madison, WI)	Ameliorates chronic idiopathic constipation; along with *B. animalis* subsp. *lactis*BB-12 help in reduction of infancy infections	(Denis et al. 2005; Fang et al. 2018)
B. bifidum	Flora Bear for Kids Renew Life	–	Exhibit anti-carcinogenic and immunomodulatory effects; suppressed allergic responses and anti-inflammatory bowel disease; eczema in infants as well as adults with irritable bowel syndrome	(Liu 2000)
B. longum MM-2	Philip's colon health capsules	Proctor & Gamble	Used in reduced gastrointestinal, immunological, and	(Christoph et al. 2018)

(continued)

Table 2.1 (continued)

Strain	Commercial product/ available form	Source	Health benefits	Reference
			infectious diseases, helps in modulation of luminal metabolism, maintains homeostatis by stabilizing gut microbiota	
Bifidobacterium breve strain Yakult	Yakult	Yakult (Tokyo, Japan)	Used in pediatrics, antimicrobial activity against pathogen; pre-obese adults body fat reductions -*B. breve* B-3	(Chen et al. 2015; Hara et al. 2003)

strains of genus in Lactococcus, *Enterococcus*, *Oenococcus*, *Pediococcus*, *Streptococcus,* and *Leuconostoc* consist of gram-positive, facultative anaerobic/ microaerophilic rod-shaped bacteria and have potential to convert hexose to lactic acid resulting an acid environment which inhibits/controls the growth of numerous harmful bacterial strains (Christoph et al. 2018). Hence this genus has pivotal role in health related sectors especially food, human, and animal due to their characteristic properties indicating their involvement towards providing health benefits beyond the basic nutritional supplements. According to one estimate, the economic significance of Lactobacilli as probiotics is expected that by 2022 to reach a market value of $64 billion (https://www.marketsandmarkets.com/Market-Reports/probiotic-market-advanced technologies–and-global market-69.html). *L. acidophilus, L. casei, L. paracasei, L. rhamnosus, L. delbrueckii subsp. bulgaricus, L. brevis, L. johnsonii, L. plantarum, and L. fermentum* are the generalized Lactobacilli species that are used as probiotic products.

Normally, Lactobacilli colonize in human, in the vagina and gastrointestinal tract, mainly either single or together with *Bifidobacterium* another probiotic microbe. Although most of the Lactobacilli are often found in the human gastrointestinal tract, as inhabitants, they are also reside in the fermented foods and oral cavities, especially colonize and cause dental caries (Messina 1995; Najma et al. 2016). Specific lactobacilli species are also utilized in the production of yogurt, sauerkraut, pickles, cheese, sourdough, wine, etc., some of the lactobacilli species such as *Lactobacillus plantarum and Lactobacillus casei* are shown in Fig. 2.2(a), (b).

2.5.2 Probiotic Properties of the Genus Bifidobacteria

Bifidobacteria also belong to group lactic acid bacteria (LAB) and are the major constituents of the gastrointestinal (GI) tract microflora of animals as well as

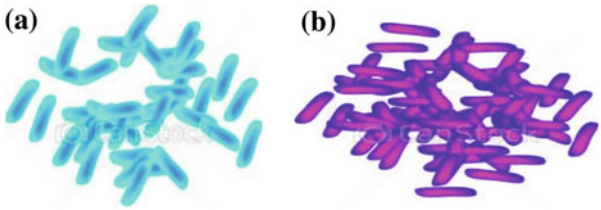

Fig. 2.2 (**a**) *Lactobacillus plantarum.* (**b**) *Lactobacillus casei*

humans. These groups of microbes are characterized with gram-positive nature, non-motile, anaerobic, saccharolytic, and endosymbiotic inhabitants of the vagina and GI tract of mammals (Brenner and Chey 2009). They are used as "probiotics," to restore normal bacteria, particularly when a person treated with antibiotics, which destroy disease-causing bacteria, including normal bacteria in the GI (gastrointestinal) and urinary tracts. Bifidobacteria have a mutual or a symbiotic association with host and help host nutrition process in metabolizing stomach undigested carbohydrates (dietary fiber, starch, galactan, sucrose, amylopectin, pullulan, etc.) in which process the bifidobacteria selectively stimulated and colonized the intestinal tract and are effective against in treating constipation. Some of the commercially available probiotic bifidobacterial strains are reported in (Table. 2.1). These probiotic microbiota have also demonstrated effectiveness against travelers as well as, antibiotic-associated diarrhea, food allergies, cholesterol-lowering capacities, preserving remission of gut inflammation, in treating necrotizing enterocolitis especially in newborns and ulcerative colitis prevention in addition to reduction of radiation induced diarrhea, lowering the development of eczema risk (Chen et al. 2007; Liu 2000).

Recently, clinical efficiency of *Bifidobacterium longum* subsp *longum* BB536 showed multifunctional probiotic role mainly in alleviating gastrointestinal, immunological and infectious diseases by modulating the luminal metabolism (Chang et al. 2008) by fine tuning homeostatic balance in gut microbiota as shown in (Fig. 2.3).

2.5.3 Probiotic Properties of the Genus Yeast

Saccharomyces boulardii is the most-evaluated probiotic yeast sp especially in successful employment in treating of multiple GI disorders. The administration of lyophilized form of probiotic microbes is found to be effective in reducing the span of the disease, irrespective of its cause. It is also reported to prevent and relapses of inflammatory bowel disease, as well as moderate ulcerative colitis (Dufresne and Farnworth 2000). However, the use of *S. boulardii* in reduction of *C. difficile* infection relapse is still under dispute. The probiotic functional role of yeast example *Saccharomyces cerevisiae as* shown in (Fig. 2.4) is attributed to yeast mediated enhanced growth of other probiotic microbes under a specific environment (acidic) conditions. This is based on the evidence that *Saccharomyces cerevisiae* EC-1118

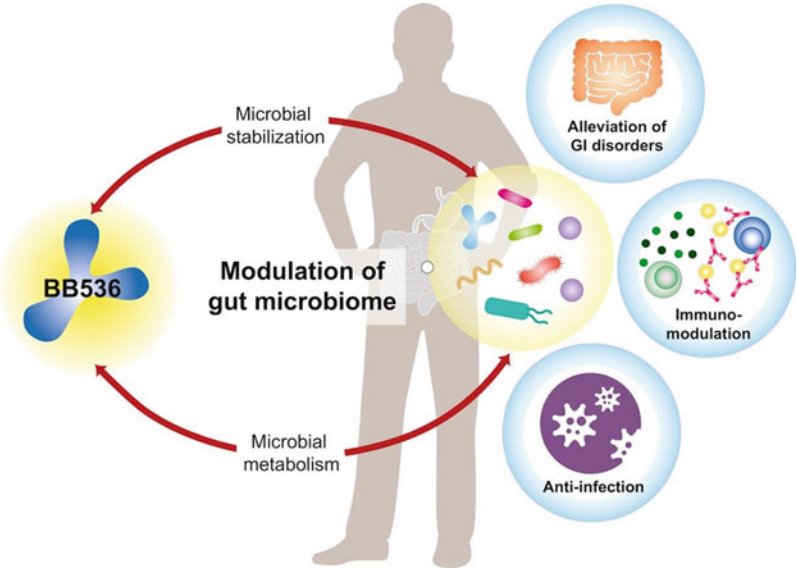

Fig. 2.3 Multifunctional probiotic role of *Bifidobacterium longum* subsp. *longum* BB536 in gastrointestinal tract (Reproduced from Chyn et al. 2019)

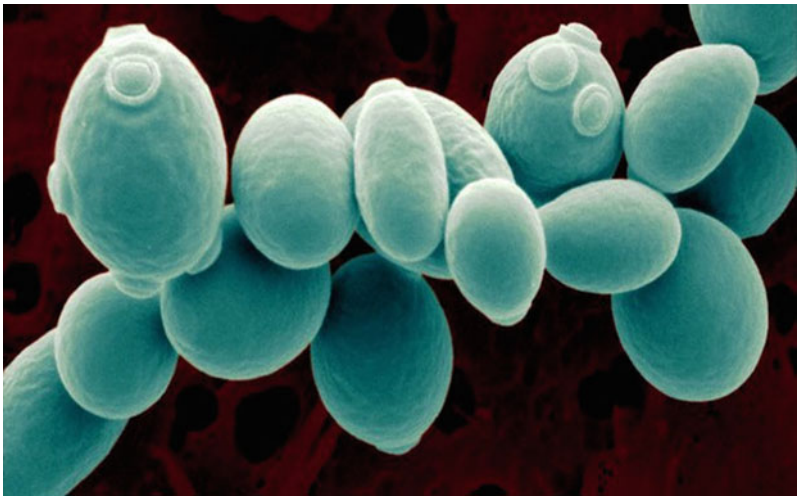

Fig. 2.4 Yeast species—*Saccharomyces cerevisiae*

influenced significantly the viability of a probiotic strain, *Lactobacillus rhamnosus* HN001 at pH 2.5 to 4.0. Among different probiotic yeast strains *Torulaspora delbrueckii*, *Debaryomyces hansenii, Yarrowia lipolytica*, *Kluyveromyces lactis, Kluyveromyces marxianus,* and *Kluyveromyces lodderae* (Rima et al. 2012) depict

high acid tolerance as well as strong antagonistic influence against pathogenic microbiota. This genus reported causes localized infections mostly in immune compromised patients, in spite of an outstanding track record of secure use as probiotics (Elsa et al. 2017; Messina 1995) hence need a precautionary advice before their use as probiotics.

2.6 Intestinal Microbiota Vs Health and Disease

In general, microbes are normal cohabitants with humans, many tissues such as skin, vaginal tract, respiratory and gastrointestinal (GI) tract. Microbes habitat themselves throughout the GI-tract mainly reside in the colon which are higher (40 trillion) than even human (30 trillion) cells. This bacterial population is a consortium comprising up to 1000 species in the human gut microbiome weighing around 1–2 Kg similar to the weight of normal brain, and each of them has vital/specific role in providing health benefits to human (Chang et al. 2008). The gut microbiome contact reported to start on the body while the baby passing through your mother's birth canal. Contradicting evidences suggest that babies may come in contact with the microbes while the baby is inside the womb, for example, bifidobacteria that first begin to grow inside the baby's intestines and digest the healthy sugars in breast milk that are important for growth (Aagaard et al. 2012). Gut microbiome embark on to diversification as baby grows based on our food habits. The human probiotic microbes, mainly the gut microbial community is generally considered as an "essential organ." This is evidenced from the reports that the gut microbial consortia is a part of primary human metabolic processes which transform the metabolic phenotype, by regulating epithelial development and influencing innate immunity. The imbalance of probiotics may lead to chronic diseases like obesity, inflammatory bowel disease (Binna et al. 2016; Chyn et al. 2019) metabolic syndrome, diabetes mellitus, atherosclerosis, alcoholic liver disease, cirrhosis, nonalcoholic fatty liver disease, and hepatocellular carcinoma (Hara et al. 2003). Though scanty information is available on molecular mechanistic evidences on probiotic involvement in improving human health, however, in the recent years, an incredible quantity of confirmation denoted a crucial role of the human microbiota in human health versus disease via numerous mechanisms (Chang et al. 2008).

This is because, microbes inhabit the length of the GI tract residing largely in the colon to enhance energy drawing out of food, improve nutrient yield, and modify appetite signaling. This microbiota equipped with far more diverse metabolic genes (~ 150 times more genes than the entire human genome) indicating its diversity in existence and offers its host with exclusive and precise biocatalysts and biochemical pathways. Moreover, probiotic metabolic processes are known to facilitate beneficial to the host in several ways as shown in (Table 2.1), including nutrient acquisition and/or xenobiotic processing as well as metabolism of undigested carbohydrates and also the biosynthesis of vitamins (Cevikbas et al. 1994). In another study it was reported that the human microbiota also act as a physical barrier, especially in protecting the host against foreign pathogens either by competitive exclusion as

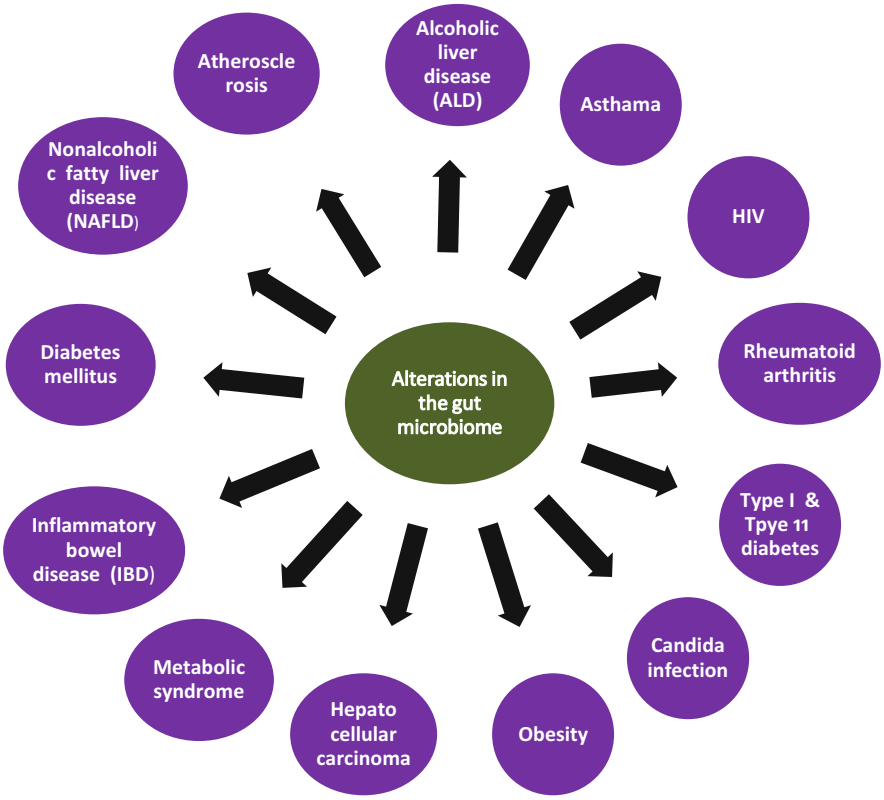

Fig. 2.5 Altered Human gastrointestinal (GI) microbiota that have been associated with chronic diseases

well as by producing antimicrobial substances (Christoph et al. 2017). After the above, the microbiota is extremely crucial in the development of the host immune system as well as intestinal mucosa. This could evidence from the fact that germfree animals possess an unusual number of numerous immune cell types showing discrepancies in local and systemic lymphoid structures, inadequately developed lymph nodes as well as spleens, and disconcerted cytokine levels. Reports on germfree animals denoted that the immune modulation functionality of the probation is initially engaged in the promotion of the immune cells maturation in addition to normal development of immune functions. Another report depicts that the vital role of microbial symbiosis in the progress of many diseases related to liver, respiratory, mental or psychological, GI malignancy, metabolic disorders, and autoimmune diseases as shown in (Fig. 2.5).

2.7 Mechanism of Action of Specific Stains of Bacterial Probiotics

Probiotic organisms lead to health improvement but mechanisms are diverse, heterogeneous, and strain specific, invitro studies on animal models reported that probiotics improve the barrier function of gut mucosa (Fig. 2.6). Stetinova et al. (2010) revealed that *Lactobacillus* and *Bifidobacterium* strains sustain on the structural components gut and produce metabolites that motivate epithelial cell signaling pathways (Madsen 2012; Makarova et al. 2006). Based on their study it was concluded that the activated B cells mediated nuclear factor of Kappa-Light-Chain-Enhancer (NF-kB), production pathway is altered by probiotics at several levels whose influences are noticed on I Kappa B protein (IKB) degradation as well as ubiquitination and proteasome activity along with nuclear-cytoplasmic movement of RelA via PPAR-gamma mediated pathway. Madsen (2012) revealed that in vivo and in vitro studies depicted that probiotic strains, *S. thermophilus* and *L. Acidophilus*, alter tight-junction protein expression and their localization. *Lactobacillus plantarum* MB452, another probiotic strain has been reported to alter occludin, proteasome, tubulin, and some cytoskeleton anchoring protein expression

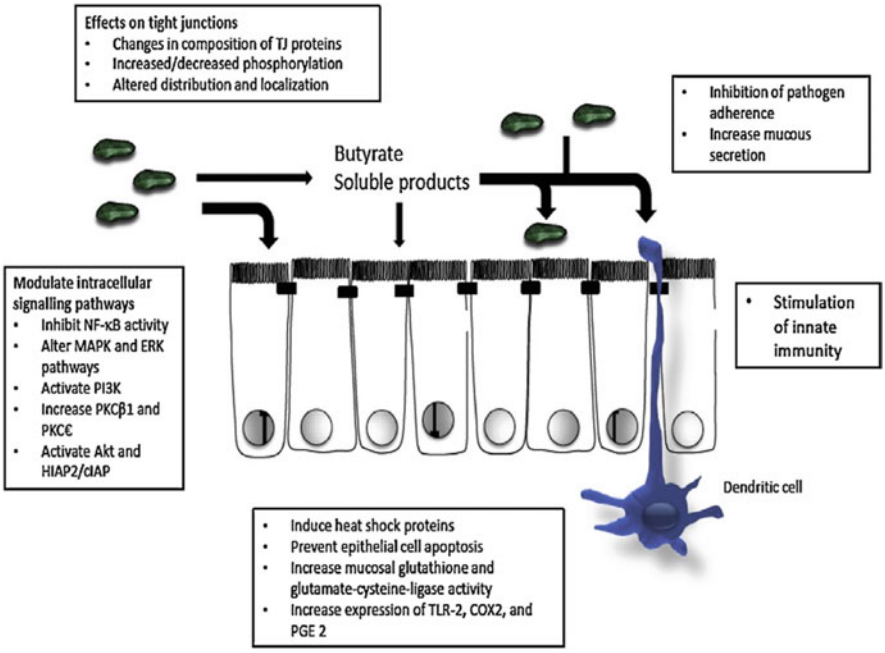

Fig. 2.6 An overview of mechanisms involved in the probiotic—induced enhancement of epithelial barrier function. These include direct modulation of epithelial cell signaling Pathways and tight junctions, as well as effects on microbial ecology, innate and adaptive immune function (Reproduced from Madsen 2012)

levels at the gene level (Baohong et al. 2017). Some probiotic strains improve cytoprotective molecule such as heat-shock protein production, which enhances gut barrier function while some avert cytokine and oxidant-induced epithelial damage and promote cell survival (Hennequin et al. 2000). Probiotics also know to modulate the functions of the immune system. This was reported by (Breidt et al. 2013) while working with *L. acidophilus* noticed that this probiotic microbe modulates toll-like receptors as well as proteins of proteoglycan in recognition of enterocytes and activates dendritic cells. This further stimulates lymphocytes T-helper 1 which triggers lymphocytes T-helper 1 cytokines and repress lymphocyte T-helper 2 responses provoking the atopic conditions (This led to decreased skin sensitivity in children and reduced disorders (eczema)). Probiotics also modulate the immune system by suppressing the growth of pathogenic bacteria through synthesizing antibiotic, bacteriocins. Probiotics, especially *B. infantis* Y1, *L. acidophilus* MB 443, *L. plantarum* MB 452, *L. paracasei* MB 451, *L. bulgaricus* MB453 reported to also produce short-chain fatty acids (SCFAs) which reduce the pH of the gut, which selectively favor the growth of advantageous microbes (Baohong et al. 2017) and inhibit pathogenic bacteria from attaching to cell walls of the gut. A few strains of Lactobacilli produce human mucus binding pili through which promote colonization.

2.8 Therapeutic Effects of Probiotics

2.8.1 Acute Gastroenteritis

The best studied example is the treatment of acute infantile gastroenteritis using probiotics in various populations. The European society for paediatric gastroenterology, hepatology, and the nutrition working group reported that the patients suffering with acute rotavirus diarrhea when treated with *Lactobacillus* strain GG (ATCC 53103) as fermented milk, improved over who were supplemented with pasteurized yoghurt but not in the case of non-specific diarrhea. The effect can be the result of several facts associated with probiotic organism mediated functionalities. They may include indigenous microbiota stabilization or diminution of rotavirus shedding period, the modification of gut permeability, and increasing in IgA secreting cells. The study depicted that the administration (Dufresne and Farnworth 2000) of a probiotic prevents the evolution of rotavirus diarrhea and acute infantile diarrhea when supplemented with *Bifidobacterium bifidum* (Seockmo et al. 2016) and *Streptococcus thermophilus.* Probiotic supplementation also results in a drastic reduction in the incidence of diarrhea in malnourished Peruvians non-breastfed children. In another study, a group under (Szajewska et al. 2013) evaluated and concluded that *Lactobacillus rhamnosus* GG alter the incidence of nosocomial diarrhea (Hara et al. 2003) in comparison with placebo even though the prevalence of rotavirus infection was similar in probiotic and placebo groups.

2.8.2 Inflammatory Bowel Disease

Probiotic bacteria have the capability to stabilize the immunological barrier, especially in the gut mucosa mainly by altering the production of local pro-inflammatory cytokines and reversing a few immunological disturbances related to Crohn's disease (Dufresne and Farnworth 2000). In another study it was reported that the gut-microflora initial colonization pattern and loss of maturational signals play significant role in decreasing the intestinal surface area, changed mucosal enzyme blueprints, defects in intestine non-immunological barrier, lower inflammatory responses, altered mucosal IgA system, and oral tolerance abrogation (Guzel et al. 2011; Hara et al. 2003).

2.8.3 Probiotics in the Treatment of Atopic Dermatitis

Atopic dermatitis is one of the non-infectious inflammatory skin disorders, expanding rapidly in the twenty-first century and causes pretenses challenges to both patients and physicians. Treating such patients with probiotics is effectual in reducing the atopic dermatitis, especially in infants; however there are no concrete evidences (Hara et al. 2003). The positive effect observed for treating atopic dermatitis by the use of probiotics, especially in the late phases of pregnancy, in some incidences, may be attributed to the type of probiotic microbe, the dose size, and duration of treatment along with the method of administration. The prevalence of this disorder is noticed to be reduced with the administration of probiotics to mothers and also helps in the prevention of eczema.

2.8.4 Probiotics for Allergic Asthma

Asthma is regarded as a Th2-type inflammatory condition and clinically heterogeneous disease whose etiology is not understood well. Numerous reports revealed that the microbial consortia of the respiratory or GI tracts associated with asthma occurrence. However, it is still not clear how dysbiosis alters susceptibility to asthma and in mice it is noticed that neonatal inflammation associated with allergy is reversed by the shift of lung microbiome to bacteroides via gamma proteobacteria and Firmicutes. Probiotics mediated immunoregulatory may be attributed to microbiome secreted short-chain fatty acids (SCFAs) most specific to butyrate followed by propionate through epithelial integrity and homeostasis which need to reassure with adults (Sharma and Im 2018).

2.8.5 Probiotics for Food Allergy and Food-Induced Anaphylaxis

Food allergy is mainly due to cellular mediators associated inflammation. Cellular mediators are produced because of hypersensitivity reactions mediated through IgE

antibodies. Food allergy may also occur due to failure of oral tolerance mainly observed in infants (Denis et al. 2005). Oral tolerance linked with immunological hyporesponsiveness towards gut-microflora and also to dietary antigens. Intestinal antigens promote CD4+ Foxp3+ Tregs while epithelial cells generate the inflammatory agents like trio, thymic stromal lympho-protein, IL-33 and IL-25 causing immune response (Tomita and Maeda 2015). It is reported that intestinal probiome can promote oral tolerance leading to sensitization to food antigens (Ricci et al. 2017). In another study, it is noticed that *Clostridia* strains suppressed sensitization towards food allergens (Ibarra et al. 2018), IL-4 receptor of food allergy prone alpha chain mutant depicted a discrete microbial signature of probiotic consortia of *Lachnospiraceae, Lactobacillaceae, Rikenellaceae, and Porphyromonadaceae.*

2.8.6 Probiotics in Treatment of *Candida* Infection

Candida is a fungus that generally inhabits the mouth, throat, GI tract, and vagina and its outgrowth is controlled by the host. Imbalance in host immunity turns *Candida* to become opportunistic and initiate the proliferation mycelia as well as rhizoids that enter the mucosal membranes, resulting microscopic breaks (leaky gut) gastrointestinal tract mucosal boundary. This enhances antigens (toxic acetaldehyde byproducts) led incomplete digestion of dietary proteins which leak into the bloodstream creating antibody release and also inflammation (Inoue et al. 2007). Leaky gut syndrome is poorly understood, however noticed to cause allergic responses, depression, agitation, joint and connective tissue inflammation, headaches, irritable bowel syndrome (IBS), fatigue, and skin problems as well as autoimmune (Crohn's) disease (Dufresne and Farnworth 2000), and rheumatoid arthritis when continued long term (Jung et al. 2004).

Probiotic strains, especially Lactobacilli and Bifidobacteria suppress/inhibit the *Candida* species growth mainly in the alimentary tract and vagina as shown in (Fig. 2.7) and also inhibit the adherence of *Candida* sp. to epithelial surfaces (Jamie et al. 2015). The probable explanation may be for anti-Candida activity of the probiotics (Pavlova et al. 2002) could be either or many of the following nutritional competition, alteration of receptors of *Candida* sp., adhesions on epithelial cells, anti-Candida compounds production, enhanced intestinal peristalsis and epithelial cell renewal rates, altering of local pH as well as oxidation-reduction potential. It is also thought that the inhibition of *Candida* sp. is associated with probiotic microbe stimulated host innate and acquired immune systems, hence probiotics considered as primary defense against mucosal and systemic candidiasis of vaginal and alimentary tracks (Aagaard et al. 2012) In fact, reestablishing the probiotic flora, especially with a*cidophilus* and bifidobacteria in the intestinal track and consumption of healthy fiber diet help in restoration of microbial flora balance will also reduce *Candida* assisted disorders.

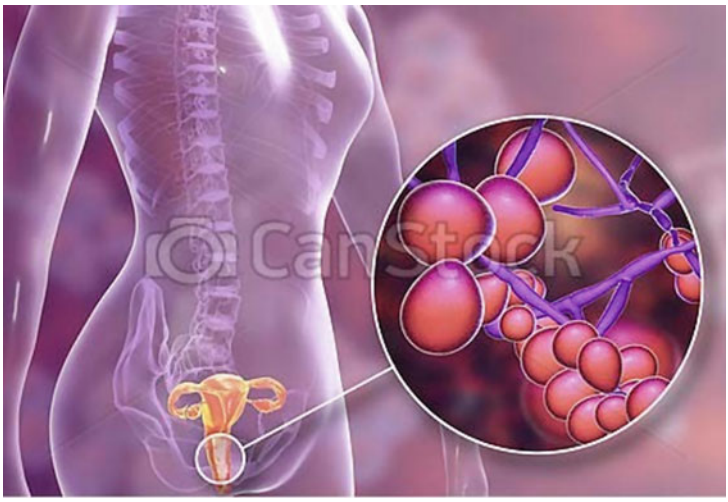

Fig. 2.7 *Candida* infection

2.8.7 Probiotics in Treatment of Colic Infection

Several literature reports are available in treatment of colic infection by *Lactobacillus reuteri* DSM17938 (Chau et al. 2015; Savino et al. 2010) and its conflicting nature (Teemu et al. 2011) based on data meta-analysis (Sung et al. 2013, 2014) colon cancer with specific symptoms like change in your bowel habits, rectal bleeding, abdominal discomfort, fatigue, weight loss, (Ohara et al. 2010), etc. It was well reported that the above symptoms are reduced upon consumption probiotic strains *L. plantarum, L. acidophilus*, and *B. longum* at a high dosage (Liu et al. 2011) which regulate the intestinal enzymes like β-glucosidase, β-glucuronidase, nitrate reductase, azoreductase, and 7-α-dehydroxylase followed by reduced production of carcinogens (Savino et al. 2016). Schreck Bird aglycones (Schreck et al. 2017), phenols, cresols, ammonia, and N-nitroso compounds from aromatic hydrocarbons/amines and their interaction with cell metabolism (Szajewska et al. 2013) and nucleic acid as well as associated cytotoxic and genotoxic effects (Hatakka et al. 2008) It is also reported that a few probiotics manipulate the immune response by activating phagocytes leading to immune-vigilance maintenance and elimination of cancer cells at primitive stage (Galdeano and Perdigón 2007; Gayathri and Rashmi 2016) which is also strain and dose dependent process (Teemu et al. 2011).

2.8.8 Probiotics in Treatment Enteric Colitis

It is a diarrheal defecation**,** an inflammation of the digestive tract, involving enteritis of the small intestine and colitis of the colon caused by several microbes, also can be regulated with probiotics (Neu and Walker 2011). Necrotizing enterocolitis, most

commonly seen in premature infants, is because of microbiota influenced intestinal homeostasis characterized with genetically predisposition, immature intestinal barrier, microvascular tone disequilibrium, and abnormal microbial gut colonization (Shiou et al. 2013) Hence, treating with probiotic microbes belongs to genus, Actinobacteria, Bacteroides, and Proteobacteria observed to improve epithelial barrier function, exclusion of pathogens, direct anti- inflammation effects, and enhanced secretary immunoglobulin A (sIgA) levels (Perdigon et al. 1995; Sartor 2004; Venturi 1999). Antibiotic enterocolitis, as name indicates, associated with antimicrobial functional properties of antibiotics in altered carbohydrate metabolism (Christoph et al. 2018) or reduction/disruption of intestinal microflora protective barrier assisted growth of opportunistic pathogenic microbial (*C. difficile, Salmonella, Staphylococcus aureus*, or *Clostridium perfringens*) growth and associated effects (Franqois et al. 1991). A concrete mechanism of action of probiotic-induced reduction enterocolitis is yet to be identified; however, upregulation of antitoxin A (Anabrees et al. 2013) secretory immunoglobulin is noticed with administration of *S. boulardii* (Amir et al. 2001) and direct inhibition of *C. difficile* toxin A binding to the epithelium (Charalabos et al. 1993) or *L. rhamnosus* associated increase of gut mucin (Catherine et al. 2008) or colonic water absorption. Comprehensive reviews by Oelschlaeger (2009) may be viewed for detailed information on mechanism, properties, treatment, etc.

2.8.9 Probiotics in Treatment of *H. pylori* Infection

H. pylori, a bacterium, grows in the digestive tract and attacks the stomach lining leading to chronic gastritis, peptic ulcer, and gastric adenocarcinoma (Kuipers 1997) in addition to extra-gastric disorders like mucosa-associated lymphoid tissue lymphoma (MALT), idiopathic thrombocytopenic purpura, vitamin B12 deficiency, and iron deficiency (Kuipers 1997). Probiotic mediated antagonistic effect towards *H. pylori* is reported in literature (Cekin et al. 2017; Kafshdooz et al. 2017); however, the exact mechanism is unclear. Antagonistic impact is known to be influenced by type of probiotic strain and its community as well as density. It was described that the antibacterial compounds such as bacteriocins, hydrogen peroxide, lactic and acetic acids produced by probiotic organisms or themselves working as adjuvants leading to inhibition/eradication of *H. pylori* or probiotics mediated delta over baseline (DOB). DOB reduction occurs through urease regulation (Salas Jara et al. 2016) by decreasing the attachment of *H. pylori* to the gastric mucosa, suppressing the *H. pylori* density (Imase et al. 2007).

2.8.10 Probiotics in Treatment of Celiac Disease (CD)

Celiac disease is linked an autoimmune response resulting in impairment of intestinal absorption mainly due to gluten ingestion (D'arienzo et al. 2011) because of the imbalance between beneficial and pathogenic microorganisms like *Salmonella, Shigella, and Klebsiella* in intestinal microbiota with helpful bacteria. Hence,

intestinal recovery of probiotic strength mainly by strains which digest gluten peptides would be the best treatment (Greco et al. 2011; Moraes et al. 2014) though main treatment for CD is the total exclusion of gluten in the diet (Moraes et al. 2014) suggested that three ways of probiotics use in CD treatment; (i) supplementation with microorganisms for CD patients, (ii) the use of glutan hydrolyzing probiotics in food production and administration of enzymes both for patients and in food production (Nóvoa Medina et al. 2008). *L. fermentum* and *B. lactis* observed to reduce the gliadin toxicity in intestinal cells (Kaukinen et al. 2014) in two different mechanism suggesting strain specificity in CD treatment. Both in the active and asymptomatic group CD groups an increase in TNF-α production and expression of CD86 in PBMC was observed which can be reversed by probiotic *Bifidobacterium longum* and *Bifidobacterium bifidum* by increasing IL-10 synthesis.

2.8.11 Probiotics in Treatment of Ulcerative Colitis (UC)

UC is known to cause by genetic mutation by aggressive luminal bacteria to initiate a mucosal inflammatory that never terminates, hence, changing existing luminal bacteria with less aggressive is major treatment. Non-pathogenic *E. coli,* supplementation is noticed to be as effective as mesalamine (drug used to treat UC) (Stetinova et al. 2010) in preventing relapse (Kruis 2004). Preliminary studies suggest that combination of eight different probiotics maintains remission and reduces active inflammation effectively (Chau et al. 2015; D'arienzo et al. 2011) while rate of relapse was observed with Bifidobacteria-fermented milk (Ishikawa 2003); however, induction of remission in small population was noticed with supplementation of *Saccharomyces boulardii* (Guslandi et al. 2003; Venturi 1999).

2.8.12 Probiotic-Rich Foods for Gut Health

Gut health, the balance of beneficial microorganisms that live in the digestive tract, is vital for physical and mental health, immunity, and more (Bourdichon et al. 2012) which is possible with foods like Kombucha (Chang et al. 2008; Dylag et al. 2014), Yogurt (Astrup 2014; Kaburagi et al. 2007), Milk kefir (Bourdichon et al. 2012; Dufresne and Farnworth 2000; Najma et al. 2016), Korean kimchi (Baohong et al. 2017; Greco et al. 2011), etc. Probiotics on one side improve digestion and another side boosts cognitive function and immunity, provides minerals mainly improve bone density, helps in treat bowel diseases and fight allergies, as well as destroy harmful microbes in the gut. Moreover, gut microbiota has vital influence on the gut–brain communication, behavior, and mood control as well as chronic fatigue syndrome. In addition, cabbage based on the kimchi/sauerkraut helps fight cancer due to the presence of increased amounts of glucosinolate (Baohong et al. 2017; Kang et al. 2016; Lira-Junior and Boström 2018). While lactose based fermented dairy foods (kefir, yogurt, and cottage cheese (Adriano Gomes et al. 2009) help in lactose intolerance (Astrup 2014). Fermented foods as shown in (Table 2.2) are well

Table 2.2 Potential health benefits of fermented foods

Fermented foods	Product form	Probiotic -starter cultures used	Source	Potential Health benefits	Reference
Kombucha	Beverage	Symbiotic culture of bacteria and yeasts	Probiotics, enzymes, amino acids, polyphenols, organic acids, ethanol, glucuronic acid, glycerol, lactic acid, usnic acid (a hepatotoxin), B-vitamins, and vitamin C	Reduction in cholesterol levels and blood pressure, cancer propagation and improvement of liver, immune systems and gastrointestinal functions	(Chang et al. 2008; Dylag et al. 2014; Markowiak and Śliżewska 2017 ; Teoh et al. 2004)
Yogurt	Dairy	*Lactobacillus bulgaricus* and *Streptococcus thermophilus*	Protein, fat, carbohydrates, vitaminB_{12}, riboflavin, phosphorus, and selenium	Lactose intolerance, constipation, diarrheal diseases, colon cancer, inflammatory bowel disease, Helicobacter pylori infection, and allergies	(Astrup 2014 ; Chen et al. 2015; Christoph et al. 2017; Guzel et al. 2011; Kaburagi et al. 2007; Ourtin and Rul 2003; Oskar et al. 2004)
Milk kefir	Dairy	*Bifidobacterium sp., Lactobacillus sp. and Saccharomyces boulardii*	Dietary minerals, vitamins, essential amino acids, and conjugated linoleic acid	Antitumor, antifungal, antibacterial properties; Immunomodulation or epithelium protection; Anti- inflammatory; Healing; Antioxidant	(Bourdichon et al. 2012; Brenner and Chey 2009; Dufresne and Farnworth 2000; Kuipers 1997; Lorenzo et al. 2014; Nardis et al. 2013; Rodrigues et al. 2005a, b; Serafini et al. 2014; Thygesen et al. 2012; Tomaro et al. 2014; Vinderola et al. 2006; Wang et al. 2008)

Korean kimchi	Fermented vegetables	*Weissella Siberia*, *Bacillus mycoides*, *B. pseudomycoides, B. subtilis, Lactobacillus brevis, Lb. curvatus*, *Lb. kimchii*, *Lb. parabrevis*, *Lb. pentosus*, *Lb. plantarum*, *Lb. sakei*, *Lb. spicheri, Lactococcus carnosus, Lc. gelidum*, *Lc. lactis, Leuconostoc carnosum, Ln. citreum*, *Ln. gasicomitatum*, *Ln. gelidum*, *Ln. holzapfelii, Ln.inhae*, *Ln. kimchii, Ln. lactis*, *Ln. mesenteroides*, *Serratia marcescens*, *Weissella cibaria*, *W. confusa, W. kandleri*, *W.kimchii. W.koreensis*, *and W. soli*	Vitamin A, thiamine (B_1), riboflavin (B_2), calcium, and iron	Probiotic function; Antiinflammatory; Anticancer; Antibacterial; Immune system promotion; Cholesterol reduction; Antiatherosclerosis.	(Breidt et al. 2013; Fang et al. 2018; Gilliland and Speck 1977; Greco et al. 2011; Roma et al. 2012)
Miso	Paste: soybeans, alone or in combination with barley, brown rice or other grains	*Aspergillus oryzae* or *Saccharomyces rouxii.*	B-vitamins, vitamins E, K and folic acid and protein	Controls Blood Pressure and Heart Rate	(Gómez et al. 2017; Taverniti et al. 2013)
Sauerkraut	Traditional vegetable	*Leuconostoc mesenteroide; Lactobacillus plantarum, and Lactobacillus brevis.*	Vitamin B6, Folic acid, Vitamin A, B-Carotene, A-Carotene, Vitamin E, Vitamin K, Lipids	Antioxidant activity, anti-inflammatory, anticancerous properties; Prevents intestinal infections.	(Bean et al. 2017; Kang et al. 2016; Liu et al. 2015; Peñas et al. 2017; Saloheimo 2005)

(continued)

Table 2.2 (continued)

Fermented foods	Product form	Probiotic -starter cultures used	Source	Potential Health benefits	Reference
Tempeh	Traditional art of soybean	*Rhizopus oligosporus; Rhizopus oryzae; R. microspores; Micrococcus; Arthrobacter sp*	Riboflavin, niacin, vitamin B6, and vitamin B12, vitamin E	Antioxidant; Radical-scavenging activity; Anti-diarrheal agent, prevent and treat chronic degenerative diseases, cancer, heart disease, Osteoporosis and postmenopausal reproductive system and bone health	(Guslandi et al. 2000; Hennequin et al. 2000; Jamie et al. 2015)
Natto	Functional soya product	*Bacillus natto*	Calcium, vitamin E, vitamin K2	Antibacterial, blood coagulation and osteogenesis	(Aloysius et al. 2015; Rijkers et al. 2011)
Fresh Cheese	Dairy	*Lactobacillus salivarius*	Casein, fat, and whey proteins	Immunomodulating capacity in mice, Probiotic food carrier	(Adriano Gomes et al. 2009; Reid 2012)

known for increased antibody levels, regulation appetite, reduced sugar levels, and help fight carb cravings (Baohong et al. 2017).

2.9 Conclusion

Probiotics play critical role in maintenance of gastrointestinal track health and or in preventing disorders in addition to regulation of nutrition, metabolism, physiology and immune response of human being. Specific gut microbial flora belongs to *Lactobacillus*, *Bifidobacterium,* and some yeast strains and their ratio controls the health status of particular person and prevents several inflammatory, allergic, and ulcer related diseases in spectrum of all aged persons including babies which can be avoided or prevented by supplementation of either probiotic related microbial strain along with supplementation of probiotic growth enhancers or nutrient rich sources to maintain the healthy gut specifically and overall health in general.

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Probiotics in the Prevention of Infant Infection

3

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Abstract

One of the most studied neonatal interventions includes the role of probiotics in treating infant infections. The use of probiotics in the pediatric field as an emerging area has been discussed in this chapter. The major focus is on the probiotics that are employed at both commercial and research levels for the prevention of various diseases and infections in infants. Major infections affecting an infant's health have also been discussed. The importance of infant formula as a suitable medium for supplementation has been discussed along with a major focus on the various types of the formula used, its viability, and commercial application. The significance of consumer perception in market stability and the market potential of the intervention together with the challenges for commercialization at a broader level was acknowledged. Along with this, the future application of synbiotic infant formula for the prevention of infection has also been referred. The importance of safety and cost along with the knowledge of adverse side effects of such interventions are also highlighted precisely. Furthermore, discussion on the future scope like addressing the safety issues related to probiotic-based foods, primarily by enlightening the area of paraprobiotics, risks associated with antibiotic resistance in preterm infants was also done.

Keywords

Neonatal · Pediatric · Supplementation · Infant formula · Synbiotics · Paraprobiotics

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3.1 Introduction

Infections are the origin and the primary reason for death and diseases among newborns in low- and middle-income countries (Coffey and Brown 2017; Duby et al. 2019; Tort and Garay 2019). Infants are more prone to the threat of diseases and infections because the major parts of their immune systems are not in a fully functional mode and still developing. Such conditions are majorly observed in premature babies (born at less than 37 weeks of gestation) and those with low birth weight (Walker et al. 2019). In response to the development of infections, inflammation, and compromised blood circulation (sepsis), this eventually results in tissue and organ injuries and growth retardation. Inadequate dietary patterns, genetic traits, environment and socioeconomic levels in society are the major causes for the development of infections in infants like gastrointestinal infections, upper respiratory tract infections, allergies, infant colic, and necrotizing enterocolitis (Tancredi 2017). Breastfeeding exclusively or artificial formula feeding for a minimum period of first 4 months is the most preferred method for prevention in cases of infections (Radzewicz et al. 2018). However, the incorporation of probiotics in the feeding formula has shown its worth in the pediatric field to serve the purpose of prevention and treatment of such infections in the most cost-effective way possible.

A newborn baby has a sterile intestine. During the process of birth and in the initial days of the baby's life, the gut is initially colonized with mainly *Enterobacteria*. The microflora develops rapidly after birth where a proportion of microbial growth is also contributed by genetic factors, delivery type (vaginal or cesarean), mother's microbiota, feeding type, and external surroundings. Mostly in breast-fed neonates, the *Bifidobacteria* counts to increase rapidly up to 90% of the total microflora in the intestine. While on the other hand *Lactobacilli* and *Bacteroides* grow slowly, and *Enterobacteria* eventually tends to decrease in number. While in the case of formula-fed infants, they mostly constitute coliforms and *Bacteroides*, with *Bifidobacteria* in less amount. Following the weaning process, the gut microbiota of children eventually starts resembling that of adults, with majorly *Fusobacterium*, *Veillonella*, and *Bacteroides* in higher numbers (Saavedra 2007; Sarkar et al. 2017). Because of the presence of such complicated and constantly modifying microflora composition in infants, the microbe-gut reactions and their effect on immune response can thereby give a significant hypothesis for using probiotics in the pediatric group against various infections.

The present chapter discusses the importance of probiotics in infant health. It also focuses on the major probiotics employed at both commercial and research levels for the prevention of various diseases and infections in infants. The mechanism behind the working of probiotics in the infant's gut is also discussed. The importance of infant formula as a suitable medium for supplementation has been discussed along with a major focus on the various types of the formula used, its viability, and commercial application. Along with this, the future application of synbiotic infant formula for the prevention of infection has also been referred. The importance of safety and cost along with the knowledge of adverse side effects of such interventions are also highlighted precisely.

3.2 Major Probiotics for Prevention of Infant Infections

Infants are always exposed to health-related risks because of the exposure to the environment, antibiotics, nosocomial exposures to disease-causing organisms, as well as alterations in typical exposure to breast milk. The two best possible ways to prevent newborns from diseases and infections are by giving probiotic to the mother during the pregnancy or directly to the infants via infant feed (Radke et al. 2017). Among the two, direct intake of probiotics to infants is better as the first-hand results without any depletion rate are observed.

Recent studies have shown that *Bifidobacterium* and *Lactobacillus* impart positive effects as probiotic agents in the prevention of different infant infections and can be considered as the best suitable probiotic strains for the infant body when taken via formula, capsules or tablet. Also, the selection and use of specific probiotics is essential since safety issues also refer to the quality of commercially available probiotics. That is why for the treatment of infants, probiotics must be developed only under strict quality control conditions.

3.2.1 Bifidobacterium

Bifidobacterium is the most commonly studied and used probiotic which is also effective in normal functionality and restoration of gut homeostasis (Sarkar and Mandal 2016). However, the mechanism of action in *Bifidobacteria* differs according to the strain type. In newborn children, bifidobacterial flora starts its growth in the gut from the initial days after delivery and constitutes a major portion of the overall microbial population (Turroni et al. 2020). Under this genus, *Bifidobacterium longum* is one of the most widespread strains of bacteria harboring in the gut microbiota of healthy breast-fed infants. Some strains like *Bifidobacterium lactis* B94 and *Bifidobacterium longum* subsp. *infantis* CECT7210 have also shown a positive effect in reducing and controlling diarrhea when subjected in the form of the supplemented formula (Işlek et al. 2014; El-Soud et al. 2015; Escribano et al. 2018a). *Bifidobacterium animalis* subsp. *lactis* BB-12 tends to reduce the occurrence of respiratory infections with the supplementation of a single probiotic (Taipale et al. 2016). Clinical studies also show that *Bifidobacterium infantis* and *Bifidobacterium lactis* when supplemented to low birth weight (LBW) infants and very low birth weight (VLBW) infants via formula, improved their growth (Härtel et al. 2017; Chi et al. 2019). Certain specific strains of *Bifidobacterium* namely, *B. breve* and *B. lactis* along with *L. casei* also, have been recorded to improve the intestinal motility and decreasing the chances of necrotizing enterocolitis in VLBW infants (Braga et al. 2011; Dilli et al. 2015). Some studies also have shown that the administration of *Bifidobacterium breve* M-16 V and *Bifidobacterium longum* BB536 to infants (for 6 months after birth) may decrease the chances of eczema development/atopic dermatitis (AD) and limit the changes in the environment of the fecal microbiota of infants (Enomoto et al. 2014; Rather et al. 2016).

3.2.2 Lactobacillus

Lactobacillus is an appropriate probiotic for the treatment of diseases associated with infants and children as several investigations have shown that it is tolerated by the gut and other body parts of the subject belonging to this age profile. *Lactobacillus rhamnosus* GG, when supplemented together with extensively hydrolyzed casein formula, promotes tolerance for cow milk allergy in infants by influencing the bacterial community structure of the infant's gut (Canani et al. 2016). Such interventions can be used to develop effective strategies against food allergies based on modulation of the intestinal microbiota. *Lactobacillus reuteri* DSM 17938 has been considered as one of the most vastly analyzed probiotics in children as well as adults with effective prevention for gastrointestinal disorders. *L. reuteri* DSM 17938 may be used predominantly to breast-fed infants for dealing with infantile colic (Szajewska et al. 2013; Sung et al. 2014; Dryl and Szajewska 2018). Also, *L. reuteri* DSM 17938 together with *L. fermentum* CECT5716 has shown positive results against the upper respiratory tract and gastrointestinal infections in infants allowing easy gastric emptying and diminished frequency of regurgitation (Wang et al. 2016b; Laursen and Hojsak 2018). *L. reuteri* DSM 17938 supplementation also reported to prevent the feeding intolerance in bottle-fed stable preterm newborns and enhancing their gut motor and immune functioning (Indrio et al. 2017). Implementation of these probiotics may thereby help to reduce the cost of the health care service. Recent studies have also suggested that supplementing preterm infants with *L. reuteri* holds the potential to reduce the risk of late-onset sepsis (LOS) and necrotizing enterocolitis (NEC) thereby allowing enteral nutrition (EN).(Athalye-Jape et al. 2016; Hernández-Enríquez et al. 2016). *Lactobacillus rhamnosus* GG has shown a partial impact on the development of the gut microbiome's compositional and functional aspect, in a manner that allows immune tolerance in high-risk (HR) for asthma infants. It delays the gut microbe development which is distinct but not yet permanent (Durack et al. 2017, 2018).

Either alone or in combination, *Lactobacillus* and *Bifidobacterium* have shown the most prominent results in the field of pediatrics. For example, the combination of *B. lactis* BB12 and *L. rhamnosus* GG is used to cure Type 1 diabetes (T1D) by diminishing dysbiosis in the gut of children (Groele et al. 2017). Also, the early colonization of beneficial microorganism i.e. *Lactobacilli* and *Bifidobacteria* in the intestine protect the gut from various types of diseases and infections. *Bifidobacteria* and *Lactobacilli,* because of their potential to modify the gut microbiota and thereby minimizing the risk of cancer, can be considered as the most important genera of organisms for infants (Yang et al. 2019; Fortmann et al. 2020).

3.3 General Mechanism of Probiotics on Infant Body

In the human gastrointestinal system, there is greater number of bacteria than the intestinal cells of the host that retain them. Irrespective of the presence of this huge microflora, humans do not get infected because of the immune responses. But in the

case of infants, since the immune system is still developing there are more chances of infection. Howsoever, the mechanism of action remains the same. The gut cells and intestinal bacteria (both formally present and deliberately added) interacts to carry-out protective mechanisms. There are various mechanisms of action focusing on this activity of probiotics against pathogens to prevent infectious diseases in the human body (Caffarelli et al. 2015; Cruchet et al. 2015). The mode of action of probiotics follows two ways, either direct antagonistic relation between commensals and pathogens occur for similar conditions, or indirect relation take place that enhance the immunomodulatory responses as depicted from Fig. 3.1.

3.3.1 Immunomodulation

The immunomodulatory activity is the indirect mode of action that differs based on various probiotic strains used for the prevention of different diseases. The most widely employed probiotics belong to the genus of *Lactobacillus* and *Bifidobacterium*. Probiotics and their subsequent metabolic components balance the pro-inflammatory and anti-inflammatory responses via different mechanisms as shown in Fig. 3.2 (Yahfoufi et al. 2018). Initially, it enables the transmission of antigens to the lymphocytes present at the submucosal layer, ensuring a more immediate immune reaction. This acts on gut mucosa by secretion of bioactive factors like immunoglobulins or cytokines, that normalize the permeability of intestine and reduces its chances of exposure to pathogens. Eventually, it allows efficient working of the intestinal epithelial barrier system with the help of certain metabolites such as acetate. Thereby, the components of the innate immune system such as macrophages and natural killer (NK) cells get activated by interacting with intracellular inflammatory pathway cells, dendritic cells, and toll-like receptors (TLRs). Finally, the adaptive immune system got influenced.

3.3.2 Antimicrobial Action

Antimicrobial production, which is a direct mode of action, inhibit the epithelial and mucosal adherence of pathogens, and compete for the restricted resources. The production of antimicrobial substances such as organic acids, diacetyl, H_2O_2, and antimicrobial peptides such as microcins or bacteriocins, aims in destroying specific pathogenic and competitor microbes. Also, the bacteriocins may serve as signaling peptides/quorum-sensing molecules in the intestinal environment. It can also act against common pathogens via toxin production or defensins secretion.

3.3.3 Competitive Exclusions

Competitive exclusion is a direct mode of action. The ability of adherence to the host by probiotic bacteria is a classical selection method that results in colonization that

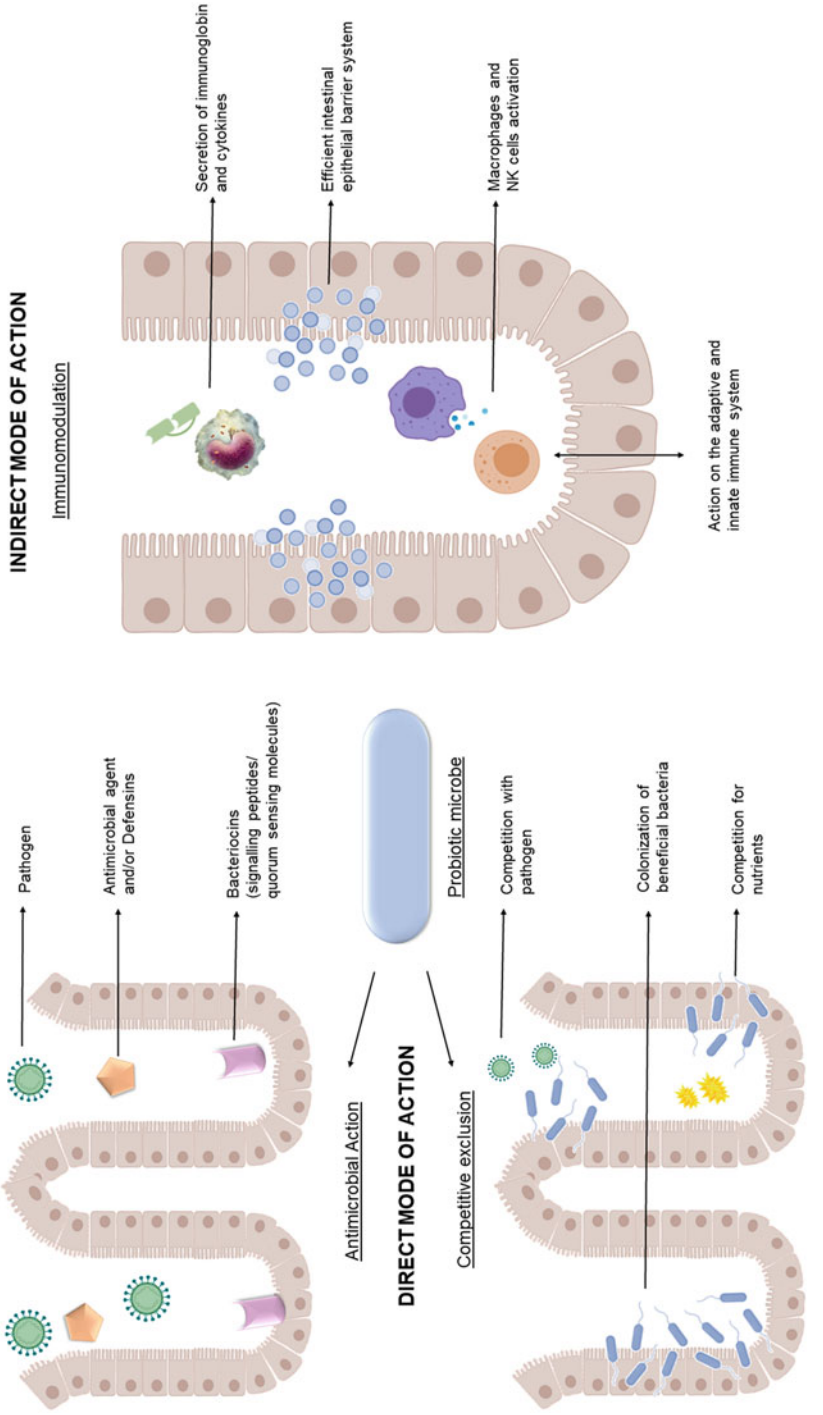

Fig. 3.1 Direct and indirect mode of action of probiotics

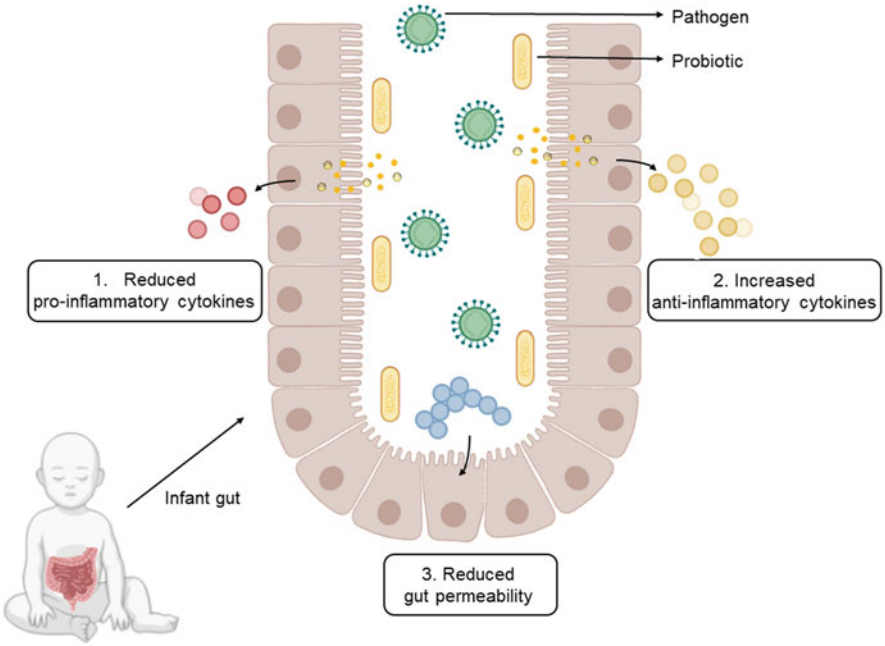

Fig. 3.2 Summary of the mechanism describing how the probiotics and their subsequent metabolic components regulate systemic immunity by (1) reducing pro-inflammatory cytokines (modulation of the innate immune response), (2) increasing the anti-inflammatory cytokines production (modifying the levels of microbial metabolites), and (3) reducing the gut permeability to release bacteria and toxins (changing the microbial population). Adapted from: (Schüller et al. 2018; Yahfoufi et al. 2018)

would eventually carry out the immunomodulatory effects and stimulate the gut barrier and metabolic functions (Monteagudo-Mera et al. 2019). The effects exerted by probiotics via competitive exclusion include either the competition with pathogens for adhering to the intestinal layer so that they can immobilize on the gut wall and resist from being removed by the action of peristalsis. Also, it may be the intestinal colonization with beneficial bacteria by occupying a niche at the expense of potentially harmful microorganisms. In direct antagonism, pathogens and commensals compete for similar nutrients and binding sites can also decrease the colonization of pathogens (Dimidi et al. 2017).

3.4 Infant Infections/Diseases and Specific Probiotics for Their Treatment

Infancy is considered a very important and sensitive stage in the building and modification of the intestinal microbiome to encounter infections and diseases in the best possible way. Initial stepwise microbial colonization along with interactions among the human host and the colonized bacteria have an ultimate impact on health

Table 3.1 Table summarizing infant infection type, preventing probiotic, and grade of recommendation (Cruchet et al. 2015)

Infection	Grade of recommendation	Probiotic for prevention
Acute infectious diarrhea	1 b	Prevention: *LGG, L. reuteri, B. lactis*
	1a 1b	*Treatment: LGG*, and *S. boulardii* *L. reuteri*
Nosocomial diarrhea	1 b	*B. lactis* Bb12*, S. thermophiles, B. bifidum,* and *LGG*
Traveler's diarrhea	1 b	*S. boulardii*
Antibiotic-associated diarrhea	1 b	*LGG* and *S. boulardii*
H. pylori	–	Not recommended
Infant colic	1 a	Colic prevention: *L. reuteri* DSM 17938
	1b	Colic treatment: *L. reuteri* DSM 17938
Necrotizing enterocolitis	1a	*B. breve*, mixtures of *Bifidobacterium* and *Streptococcus*, *LGG, L. acidophilus* and *L. reuteri* DSM 17938
Crohn's disease	–	Not recommended
Irritable bowel syndrome	2c	*LGG* and VSL#3
Allergy	–	Not recommended
Asthma and wheeze	–	Not recommended

Grade of Recommendation was assigned for pediatric gastrointestinal-related conditions, based on the updated Evidence-Based Medicine guidelines: 1a denoting systematic review (SR) of RCTs, 1b denoting individual RCT, 1c denoting SR and individual RCT, 2a denoting SR of cohort studies, 2b denoting individual cohort studies, and 2c denoting outcomes research

and disease. Right from birth, the normal gut microbiota begins to contribute to the proper functioning of the gut such as by providing protection against infections, maintaining intestinal barrier function, immune and metabolic homeostasis, and tolerance of foods (Miniello and Diaferio 2017). A slight alteration in the colonizing of microbes via disease-causing organisms holds the potential to cause several infections in early life leading to lifetime suffering. Thus, to positively modulate the gut microbial composition, an increased interest has emerged in the development of gut microbiota biomodulators such as prebiotics, probiotics, paraprobiotics, synbiotics, or postbiotics. Table 3.1 summarizes all the different infant infection types, their preventing probiotic as well as the grade of recommendation.

3.4.1 Diarrhea

In infants, different types of diarrhea are associated with the gastrointestinal malfunctioning. The majority of infections are caused by viruses, bacteria, and

parasites. The side effects observed in infants generally include abdominal pain, diarrhea, and vomiting (Gashaw et al. 2017). The four major types of diarrhea most frequently associated with infants are discussed below. The main focus is to understand the prevention or treatment methods associated with such infections.

3.4.1.1 Acute Infectious Diarrhea (AID)

It is among the most common infections associated with the pediatric group. The use of probiotics for the treatment of AID in infants is an ideal intervention as probiotics act directly on enterocytes and indirectly on the systemic immune system and intestinal microenvironment, thereby managing AID in early childhood (Lo Vecchio et al. 2019). According to researches, the most commonly used probiotic strains that reduce the symptoms and duration of AID are *L. reuteri* DSM 17938, *LGG*, and *S. boulardii* (Urbańska and Szajewska 2014; Cameron et al. 2017). A recent meta-analysis study has also investigated the efficacy of probiotics in the treatment of acute rotavirus diarrhea in infants by reviewing several studies and it concluded that the pooled estimate of the efficacy of *L. rhamnosus* GG significantly speeds down the duration of diarrhea (Ahmadi et al. 2015).

3.4.1.2 Nosocomial Diarrhea

In the case of diarrhea acquired under health care or hospital conditions (nosocomial), rotavirus is the major culprit. The main reason for this infection is prolonged hospital stay which eventually results in increased mortality rate, enhanced antimicrobial resistance, and also a monetary burden on the health care system. Studies have shown that vaccination against rotavirus decreases nosocomial diarrhea in infants (Hojsak et al. 2018). Probiotics have a significant preventive role for nosocomial diarrhea with *LGG* being the most promising one (Trivic and Hojsak 2018). To be strain-specific, the most recommended probiotic strains for the prevention of nosocomial diarrhea include *LGG* and *L reuteri* DSM 17938 having at least 10^9 (colony forming units) CFU/day, however, this dosage limit varies from one research to another (Hojsak et al. 2018).

3.4.1.3 Traveler's Diarrhea

Nowadays, in international travel or global migration, the number of children traveling (with age < 10 months) with their parents is increasing steadily. In such cases, children are at superior risk of coming in contact with regional diseases because they stay in areas with different conditions and atmosphere eventually leading to "Travelers's diarrhea" (TD) which is a common illness observed in children due to traveling. The symptoms include continuous passage of more than 3 unformed stools, with or without other symptoms like vomiting, nausea, fever, abdominal pain, which tends to develop during the first 14 days of returning from a journey (Ashkenazi and Schwartz 2019). Apart from medication and vaccines, the use of probiotics has also come in the picture recently where the administration of *Saccharomyces boulardii* CNCM 1–745 showed a significant decrease in TD.

3.4.1.4 Antibiotic-Associated Diarrhea (AAD)

It occurs in infants when they undergo antibiotic treatments excluding other etiologies. Probiotics are considered as an adjunctive treatment in case of infantile gastroenteritis with higher efficacy to reduce antibiotic-associated diarrhea in infants when compared to adults (Guarino et al. 2015). The frequent uptake of antibiotics triggers dysbiosis in infants leading to AAD (Hayes and Vargas 2016). The study also specified that a high probiotic dose is generally required (5×10^9 to 40×10^9 CFU/day) for curing the incidence of AAD in children. The most recommended probiotics and the drug of choice for the prevention of this type of diarrhea are *S. boulardii* and *LGG* (Mantegazza et al. 2018). These are effective and safe for infants and young children, both during the usage of antibiotics and up to 14 days after drug discontinuance (Wan et al. 2017).

3.4.2 *Helicobacter pylori* Infection

This infection is caused by *Helicobacter pylori* which is a gram-negative microaerophilic bacterium that colonizes the stomach (Massarrat et al. 2016; Kira and Isobe 2019). The infection is acquired in early childhood and majorly arises in case of crowded and low socioeconomic living conditions. As the infection establishes, chronic gastritis develops along with ulcers, and in rare cases mucosa-associated lymphoid tissue lymphoma and distal gastric cancer (Dror and Muhsen 2016). The identification of this infection is not as easy as the symptoms do not express widely. Also, the characteristics of this infection are different in the case of adults and infants (Yang 2016; Kori et al. 2018). Most *H. pylori*-infected children remain asymptomatic but develop chronic superficial gastritis while in the case of adults, gastric cancer (adenocarcinoma) can be easily detected. Studies have shown that even exclusive breastfeeding is not effective in preventing or facilitating this infection (Asgarshirazi et al. 2017; Chi et al. 2019). Thus, treatment by infant formula supplemented with probiotics must be employed. In a study, a complex meta-analysis was done to compare the efficacy and safety of probiotics supplemented in 14-day triple therapy in Asian pediatric patients. A positive result was obtained where *Bacillus mesenteric* together with *Clostridium butyricum* was the best combination for eradication rates of *H. pylori* while *Streptococcus faecalis* together with *B. mesenteric* and *C. butyricum* was best for reducing the incidence of total side effects. (Wen et al. 2017).

3.4.3 Infant Colic

Continuous infants crying and fuss without any evident reason lasting up to 3 hours or more per day and at least 3 days per week is the condition referred to as Infant colic. The reason for this is still unclear but the options for prevention of the same holds a big question. In a recent meta-analysis, *L. reuteri* was regarded as the most employed probiotic in the majority of treatments (Mu et al. 2018; Cui et al. 2019),

and it was found that a significant lowering in crying rate in colicky breast-fed infants was there when compared to the control (placebo) cases (Karkhaneh et al. 2019). Several types of researches have been done to evaluate the potential of probiotics in the prevention of this disease. To be strain-specific, the administration of *L. reuteri* DSM 17938 has been reported by a lot of studies to lower the crying times in breast-fed infants with infantile colic (Szajewska et al. 2013; Dryl and Szajewska 2018; Ong et al. 2019).

3.4.4 Necrotizing Enterocolitis (NEC)

Necrotizing Enterocolitis is that condition where the reaction between an immature gastrointestinal system and pathogenic microbial agents, causes colonization in the gut and then the further interactions with chemical substances from food cause morbidity, intestinal dysbiosis, and neonatal mortality in VLBW infants (Thomas et al. 2017). NEC has also been associated with the delay in the development of the nervous system and the chances of short-gut syndrome in infants. Among different preventive agents like prenatal glucocorticoids, breast milk, standardized feeding procedures, and bovine lactoferrin, the application of probiotics in reducing the risk of NEC has shown better efficacy. Several extensive reviews and data analysis, it was concluded that probiotics are an advantageous and effective medium in preventing NEC and mortality in preterm neonates. Also, the combination of probiotics belonging to the genus of *L. acidophilus* with *B. bifidum* seems to produce the major benefits (Baucells et al. 2016). Another study concluded that the administration of *L. reuteri* DSM 17938 is effective in preventing necrotizing enterocolitis (Hernández-Enríquez et al. 2016). Prophylactic administration of probiotics such as *Lactobacillus* spp. either alone or in combination with *Bifidobacterium* spp. has positive results against severe necrotizing enterocolitis majorly in the case of preterm infants (Escribano et al. 2018b).

3.4.5 Inflammatory Bowel Disease (IBD)

Pediatric Inflammatory bowel disease (PIBD) is a rapidly increasing chronic disorder that majorly includes Crohn's disease (CD), ulcerative colitis (UC), and unclassified colitis (Virta et al. 2016). In this infection, the interaction of harmful microbes with specific epithelial receptors causes inflammation which in turn is responsible for the perpetuation of the disease. Probiotics are considered as the most suitable therapeutic tools for IBD (Serena and Fasano 2019). They have shown positive effects on the constituents and working of the microbiota, production of antimicrobial agents, improvement of the barrier property, reduced intestinal permeability, and changes in the innate and adaptive immune responses (Lane et al. 2017; Guandalini and Sansotta 2019). Several researches have shown the potential of a probiotic preparation (VSL#3) for treating and preventing IBD in infants (Cheng et al. 2020).

3.4.6 Irritable Bowel Syndrome (IBS)

The condition where abdominal discomfort or pain coexist together with disturbed bowel patterns is referred to as Irritable Bowel Syndrome (IBS). Based on the predominant bowel pattern, IBS can be subclassified as constipation-predominant IBS (IBS-C), diarrhea-predominant IBS (IBS-D), mixed IBS (IBS-M), and un-subtyped IBS. Studies have also shown that patients who have previously experienced infectious gastroenteritis are more prone to the risk of IBS up to seven folds (Wouters et al. 2016; Klem et al. 2017). Other than following a regulated diet and proper medication and antibiotics, the contribution of probiotics in the prevention of IBS is also emerging. Based on the available evidence from the recent studies, it has been observed that the best treatment for IBS patients is via multi-strain probiotic with a concentration of 10^9 CFU/day (Harper et al. 2018).

3.4.7 Constipation

Functional constipation (FC) is a common disease and a significant health issue in both infants and children worldwide. The major cause of FC includes inadequate dietary patterns, consumption of fast food and low/no physical activity, child maltreatment, and obesity being the prevalent risk factors in pediatrics (Rajindrajith et al. 2016). The symptoms of constipation in infants are infrequent defecation, fecal incontinence (FI), abdominal pain, anal fissures, enuresis along with the coexistence of urinary tract infections. Beyond managing the FC via education, dietary recommendations, and antibiotics intake, application of infant formulas containing partially or extensively hydrolyzed proteins, fortified with probiotics and, or, prebiotics, can be used as anti-constipation formulas (Vandenplas et al. 2015a). Although there is very limited literature about the efficacy of probiotics in curing infant constipation, however, supplementing infant formulas with probiotics and prebiotics is regarded as safe. A study suggested that effective use of *Lactobacillus reuteri* prevented constipation in early life (Indrio et al. 2014). A meta-analysis systematic review concluded that due to the lack of sufficient evidence, the recommendation of pro-, pre- or synbiotics in the prevention of infants suffering from constipation is limited (Koppen et al. 2016). On the other hand, contrary results were shown by another study where a synbiotic infant starter formula was considered safe because it helped in reducing functional constipation, colic, and regurgitation quite significantly (Yvan Vandenplas et al. 2017). Also, this formula eventually did not hamper growth and was very well tolerated by the infant body.

3.4.8 Allergy

Allergic diseases are regarded as a load on the health care system because of the potentially life-threatening reactions, eventually leading to degraded quality of life, and association with fluctuating cost expenditure. Also, the microbial gut

composition in humans allows the development of allergies because of the potentially anti-allergenic processes occurring within. Thus, alternating these microbiota in early life via the use of probiotic supplementation can be used as an attractive alternative for the curing of allergic diseases in infants (Cuello-Garcia et al. 2015; Plummer et al. 2020). Allergy can be of any type, for example, food allergy, asthma, allergic rhinitis, and atopic dermatitis. In a study (Abrahamsson et al. 2007), probiotic *L. reuteri* was orally supplemented to infants to evaluate its effects on the prevention of IgE-associated eczema. The results showed a decreased risk of developing respiratory allergic infections in treated infants as they had limited IgE-associated eczema at 2 years of age. Similar studies have also shown that supplementation of infant food with probiotic foods holds the potential to prevent and treat the causes of allergic infections in infants.

3.4.9 Asthma and Wheeze

Developing the immune system in infants allows the growth of commensal gut bacteria which eventually leads to the disruption of gut microbiota that may contribute to immune disorders in the later life of a child. Researches have shown that uneasiness in infant gut microbiota causes the development of atopic dermatitis also called allergic eczema, which is the first step towards allergic rhinitis and asthma (Avershina et al. 2017). Other factors such as cesarean delivery, use of antibiotics, lack of breastfeeding, and nutrition deficiency increase the risk of asthma and wheeze (Azad et al. 2013). Asthma and wheeze are the diseases under respiratory tract infections (RTIs) which is one of the main health-related issues in the case of infants. In a recent systematic review of meta-analysis, it was concluded that probiotic consumption can be employed as a feasible way to reduce the incidence of RTIs in newborn and young children (Wang et al. 2016a). In a study, infant formula was administered with *L. fermentum* CECT5716 and its effects were evaluated which ultimately depicted that this particular strain may be useful for the prevention of infections associated with the upper respiratory tract (Maldonado et al. 2012). Several studies have evaluated the effect of probiotic supplementation during infancy in preventing atopic dermatitis in children concluding that interventions of mixed *Lactobacillus-Bifidobacterium* are suitable supplements contributing to reduced risk ratio (Szajewska et al. 2013; Yin et al. 2019). Another study evaluated the significant effect of prophylactic probiotics, specifically *Lactobacillus* and *Bifidobacterium* strains, on the occurrence and symptom scores of RTI in normal healthy children. The results showed that supplementation of such microbes in children with immune-competency has an adequate impact both in minimizing the incidence of RTIs and the severity of the infection symptoms (Ozen et al. 2015).

3.5 Probiotic Infant Formula: Types, Viability, and Use

Infant formula is an industrial substitute for infants aiming to imitate the nutritional composition of breast milk. Enriching infant formula with probiotics is another strategy employed by manufacturers to increasingly exploit its use. An extensive literature on the same tells that the application of probiotic enriched infant formula allows easy uptake by infants, ease in clinical testing, and enhancement in results against infections. Health-based clinical studies have shown that supplementing infant formula with *Lactobacillus fermentum* CECT 5716, which is a human milk probiotic, tends to modify infant microbiota composition in such a manner that it cure infections. The main aspect of supplementation with these ingredients is that if positive effects are seen in early life, the chances of long-term side effects in host metabolism and physiology reduces automatically. For example, in a study, the relation of early exposure of probiotics on islet autoimmunity (autoantibodies directed against insulin) was examined (Uusitalo et al. 2016). The results concluded that supplementing probiotics at an early age in newborn infants reduced the risk of type-1 diabetes mellitus (T1DM)–associated islet autoimmunity.

The selection of the best suitable matrix is thus important in infant formula. This matrix is of three types, namely liquid, powder, and ready to feed. The powder is the cheapest form of infant formula that is mixed with either water or milk before feeding. Liquid, on the other hand, is majorly present in concentrated form and must be diluted with an equal quantity of water. Ready-to-feed form of infant formula is expensive among all and does not requires mixing. Broadly, there are three distinct groups of infant formula namely, cow milk-based formula, soy-based formula and specialized formula like hypoallergenic formulas, protein hydrolysate formulas, and amino acid formulas (Canani et al. 2016; Martin et al. 2016; Radke et al. 2017). The food industry majorly involves milk or yogurt drinks as the medium for microbial supplements (Ringel-Kulka et al. 2015). Capsules and dried probiotic powders are also some other commercially available options for microbial supplements.

Ensuring the required viability of probiotic cells in the end product via food processing and also in the gut is most important for the microorganisms to enable them to attach to the final site of action in a required amount as prescribed (10^8 CFU/g). This will ultimately exert positive results to the host. Cell viability is also important in terms of marketing as manufacturers are only then allowed to make a probiotic claim for the product (Kent and Doherty 2014; Champagne et al. 2018; Sanders et al. 2018). Usually, maintaining the required count is difficult because there is an evident loss inviable cells in the route of the stomach having a low pH environment and high bile salt levels in the intestine (Sagheddu et al. 2018; Hati et al. 2019). In addition to that, food processing techniques also exert stress and the conditions experienced during storage that can also have a negative impact on the growth and viability of probiotic microbes (Das et al. 2014; Cassani et al. 2020). In a study, the bacteriological stability and viability of a probiotic mixture (*B. bifidum* and *L. acidophilus*) dispersed in various diluents (sterile water, breast milk, and formula feed for infants) was examined at temperatures of 4 °C and 21 °C. The result

showed that at 4 °C storage temperature, the probiotic can remain at a stable condition for 6 h when diluted with infant formula and sterile water, however, the probiotic survivability tends to decrease after this period. The research concluded that administration of this probiotic having sterile water as diluent can be used as an alternative for dispersion and thereafter it can be administrated in breast milk (Watkins et al. 2018). Microencapsulation is one of the most efficient techniques to provide a physical barrier to protect probiotics and to deliver them into the gut and preventing them from all sorts of ingestion, processing, and storage conditions (Martín et al. 2015; Amin et al. 2019). The concept of microencapsulation is based on the immobilization of bacteria (which is the core) into a matrix that allows structural strength until the releasing time of the cells in the intestine. The most commercially applied probiotic-containing microcapsules production methods include spray-drying, emulsion technique, and extrusion technique (Sarao and Arora 2017).

3.6 Role of Synbiotics in Infant Infections

Extrinsic components have shown maximum contribution in developing the health of the intestinal microbiome by altering the microbial composition of the infant's gut via intake of pre- and probiotics. Extensive research on the potential opportunities by this beneficial supplementation in the promotion of a healthy gut has proved that the use of probiotics and prebiotics is a smart strategy for the reduction and prevention of GI infections (El Hage et al. 2017; Cassani et al. 2020). To promote healthy growth and development with reduced infections rate in infants, pre- and probiotics are supplemented in infant formula. The major aim of this supplementation is to enhance the concentration of good microbes in the infant body as the immune system is still under development.

3.6.1 Effect of Prebiotics Supplementation

Prebiotic oligosaccharides (OS) are generally considered as a very essential constituent of breast milk which is not present in cow's milk (Bertelsen et al. 2016). The most common prebiotics added to infant formula includes galactooligosaccharides (GOS) and fructooligosaccharides (FOS). The main idea behind prebiotics addition to infant formula is to enhance the growth of probiotics in the gastrointestinal tract of infants and replicating the environment as in the case of breast-fed infants (Vandenplas et al. 2015b). Prebiotic supplementation increases the frequency of stools but generally, no results can be observed on the consistency of stool, regurgitation, the incidence of colic, and restlessness (Skórka et al. 2018; Oswari et al. 2019). Best results of prebiotics are observed when taken together with probiotics as the symbiosis allows added benefits of both components.

3.6.2 Effect of Probiotics Supplementation

With the mindset of replicating breastmilk as closely as possible and to enhance the intestinal microbiota of infants, the top most researched and employed probiotics belong to the genera *Lactobacillus* and *Bifidobacterium* (Fortmann et al. 2020). Extensive studies on these probiotics as the beneficial agents for gut health in infants have shown that formula supplemented with probiotics minimizes the chances of gastrointestinal infections, respiratory infections, infantile colic, diarrhea, NEC, constipation, and other infections. A detailed list of infant's probiotic clinical trials and their protective outcomes are presented in Table 3.2 briefly discussing the protective outcome (from different researches) of the probiotic used in a specific dosage and matrix on a particular population.

Probiotics and prebiotics in addition to infant formula tend to show a positive influence on GI microbiota composition. Though clinically significant health care advantages of pro- and prebiotics added infant formula are less, it has to be mentioned that no negative effects are reported by such formulas and maximum trials show some advantages though not always relevant. Nevertheless, the supplementation of these ingredients to infant formula allows an option for infant feeding concerning breastfeeding (Vandenplas 2016).

However, less but recent studies assessing the efficacy of synbiotic supplementation in infants have proved there are some promising results regarding the positive impact on infant health against several infections. In a study, an oral synbiotic preparation (*Lactobacillus plantarum* plus fructooligosaccharide) and its uptake significantly reduced neonatal sepsis (Panigrahi et al. 2017). Another work studied that a synbiotic yogurt drink containing *L. bulgaricus*, *S. thermophilus*, and *B. animalis* subspecies *lactis* (BB-12) together with inulin when given to children 12–48 months of age eventually reduced the fever days (Ringel-Kulka et al. 2015). A trial on the application of the synbiotic formula of *Lactobacillus, Bifidobacterium,* and fructooligosaccharide for the prevention of NEC was also carried out (Nandhini et al. 2016). The results concluded that although the intake of synbiotics did not significantly decrease the extent of severity of sepsis, NEC, or mortality, however, about 50% decrease in the occurrence of NEC of all levels in preterm infants was still reported there. Thus, enteral supplementation with probiotics, prebiotics alone, or in combination (synbiotics) has shown to be a beneficial strategy for infants when dealing with the prevention of NEC, mortality, respiratory infections, and sepsis. However, apprehension is still there regarding the doses, specific strain to be used, duration of supplementation to exhibit positive results, the cost-effectiveness, and longevity of pre- and probiotic supplementation in early life.

Table 3.2 Probiotic clinical trials in infants: protective outcomes

Patient Population	Patients No.	Probiotic used/Dosage	Source	Protective outcomes	References
Healthy infants (>3 months)	93	*B.longum* subsp. *infantis* CECT7210,10^7CFU/g, for 12 weeks	Supplemented formula	Increased IgA conc., reduced diarrhea, reduced constipation	Escribano et al. (2018a)
Newborn 1-month-old infants	109	*B. animalis* subsp. *lactis* BB-12, 10^9 CFU/g, twice a day	Test tablets	Reduced occurrence of respiratory infections, GI infection, and acute otitis media	Taipale et al. (2016)
Low birth weight (LBW) infants	97	*B. animalis* subsp. *lactis,* 10^6 CFU/g, till 14 days after birth	Supplemented formula	Improved gut health with microbiota containing beneficial bacteria	Chi et al. (2019)
Healthy infants (6–12 months)	215	*L. fermentum* CECT5716, 2×10^8 CFU/day	Supplemented formula	Reduction in GI, respiratory infections, influenza	Maldonado et al. (2012)
Infants (<6 months) with infantile colic	471	*L. reuteri* DSM 17938, 10^8 CFU/day	Supplemented formula	Reduction in crying as well as crying time	Dryl and Szajewska (2018)
Infants with regurgitation	80	*L. reuteri* DSM 17938, 2.8×10^8 CFU/powder for 4 weeks	Partially hydrolyzed whey infant formula Supplemented with starch	The gastric emptying rate increased, episodes per day of regurgitation reduced	Indrio et al. (2017)
Bottle-fed preterm newborns	79	*L. reuteri* DSM 17938, 10^8 CFU/day for 30 days.	Supplemented formula	Prevented feeding intolerance and improved gut motor and immune function development	Indrio et al. (2017)

3.7 Cost, Safety, and Adverse Effects

Probiotics are regarded as "generally recognized as safe" (GRAS) and its use is well tolerated by infants and children. Generally, the microbes belonging to Lactic acid bacteria (LAB) and *Bifidobacterium* are non-pathogenic and non-toxic traditional food-grade microbes used in the food industry. Despite the fact that probiotics are a very essential part of the gut microbiota and are contained in daily uptake foods, the doubts on safety still remains a question. Quite a few, but probiotic-related neonatal sepsis have been reported in time arising the question of safety. (Dani et al. 2015; Molinaro et al. 2016).

Several reports on neonatal probiotic sepsis have shown that probiotic safety is a serious topic and must not be taken for granted and requires continuous surveillance. Some of the most potential long-term adverse effects of probiotic supplementation in neonates include the development and transfer of antibiotic resistance, altered immune responses, and allergic sensitization (Deshpande et al. 2015). For the safety of infants and newborns, *Bifidobacteria* and *Lactobacilli* have shown a unique safety profile to be used in the field of pediatric study. The most common adverse effects associated with probiotics consumption include bloating and flatulence. *Lactobacillus* sepsis in infants with the short-bowel syndrome, *Bacillus* sepsis in case of extremely low birth weight infant and *B. breve* sepsis during post-operative omphalocele has been reported as adverse events (Mayer and Kerner 2017; Abdul Hakeem et al. 2018; Graspeuntner et al. 2019). Allergic reactions are also induced by probiotics and are considered as the side effects of its administration. The occurrence of D-lactic acidosis via probiotic metabolic activity in infants suffering from short-bowel syndrome has been observed. The cases of crying, fuss, and regurgitation in infants after adding with specific probiotic strains have also been recorded to decrease in a significant manner (Caffarelli et al. 2015). Another recent study investigated the effects of the supplementation of probiotic infant formula compared with unsupplemented ones. The probiotic-supplemented infant formulas were *Bifidobacterium lactis* Bb12, *Streptococcus thermophilus, Lactobacillus johnsonii* La1, *Bifidobacterium longum* BL999, *Lactobacillus rhamnosus* LPR, *L. rhamnosus* GG, *Lactobacillus reuteri* ATCC 55730, *L. reuteri* DSM 17938, and *Lactobacillus salivarius* CEC5713.The study concluded that the probiotic-supplemented formula given to healthy newborns does not question any safety concerns in terms of growth and also, health-based adverse effects and some clinically significant effects were also present (Vandenplas et al. 2015a). Thus, it can be observed that the incorporation of probiotics in infant formula is a potential medium to explore and apply commercially.

The intensity of safety or prevention from an infection is majorly contributed by the total daily intake of the probiotic used. According to the Food and Agriculture Organization guidelines and the studies dealing with probiotics ensure that the intake of these beneficial microbiota in viable form is between 10^8 and 10^{10}CFU/day. The most reasonable consumption of probiotic in the required "dose" can be done in the form of a tablet or capsule. But for application in the pediatric field, the most practical way of incorporation of selected probiotics can be via infant diets such as

infant formulas or weaning foods. This approach can also allow easy compliance, reduced costs, and effective results when compared with a daily supplement (Lönnerdal and Hernell 2016).

Thus, even by considering probiotics as safe, the source of opportunity for the development of bacteremia/fungemia remains there. Hence, clear details of the safety norms and toxicity levels (as per the standards) associated with the probiotic administration in case of high-risk age categories such as infants and immune-deficient patients must be initially clarified.

Probiotic supplementation in preterm neonates thus tends to exhibit a good safety profile and has minimum side effects. However, more randomized controlled trials are still emerging to understand the safety profile of supplemented probiotics for preventing infections and also as an additional treatment to prevent invasive fungal infections in preterm neonates. Best suited probiotic variety, administration time, and host selectivity are still unclear due to heterogeneity of trial design. That is why many repeated studies employing single design protocols are required and needed to demonstrate reproducibility, safety, and efficacy.

3.8 Consumer Perception, Market Potential, and Challenges for Commercialization

Consumer acceptance and perception is of utmost importance in business and market growth. It allows scope for improvement and decides whether the product should be present in the market or not. In a very recent survey on acceptance of probiotic therapy in preventing infant AAD, it was observed that about 51% of parents and 51% of clinicians said that they would use probiotics if it limits the risk of AAD by 39%. The most important outcomes to parents and pediatricians were need for hospitalization, disruption of normal daily activities, and physician revisit. They rated the curing of dehydration, duration of diarrhea, and frequency of stool as important outcomes as well (Khanpour Ardestani et al. 2019). Thus, it can be concluded that parents are now in a state to accept probiotic-based infant formulas and food products as long as it ensures an acceptable amount of positive results on infant health against infections, at the same time without causing any adverse effects.

Probiotics based food products are emerging successfully in a variety of marketing channels such as online sales. But at the same time, there arises one of the major issues related to these products concerning their safety when subjected to the commercial market. Thus, limited knowledge about the scientific evidence of the medicinal potency of such products and restricted dominance of their composition and storage life, and lack of data on side effects or supplement drug interactions may become challenging pillars for their potential in the probiotic market (Cruchet et al. 2015). Indian probiotic industry includes some famous probiotic brands that also have shown their capability globally. These brands are Amul, Nestle, Mother Dairy, and Yakult Danone along with some other minor companies and startups operating in different regions in their scope.

The major challenge in the continuous growth of the Indian probiotic industry is probably the lack of standardization in process and product development. As the industry is still growing, the absence of proper standardized parameters creates a hurdle. Lack of awareness and hesitation in acceptance of these products, particularly from the lower-middle-class population group in urban society and rural regions may act as a obstacle for the companies in their expansion strategies for the future. A possible solution for this problem may be via television advertisement campaigns, free trials, educational seminars, etc., to promote the product and aware masses about their benefits. Marketing and distribution are also a type of challenge as in the case of diverse countries like India where region-specific marketing strategies must be required. In such cases having a local sales team for the decision-making process and launching of products will help in the efficient product sale and growth in the business. The involvement of defined and renowned strategies with a positive goal can make a difference in solving this challenge. Introducing products with consumer interests, perceptions, and satisfaction reduces major challenges in a very effective way.

3.9 Conclusions and Future Scope

Probiotics are one of the most studied neonatal interventions, and their application in curing infant infections has been referred to as a "golden age." This emerging area of probiotics and their use in the pediatric field have been discussed throughout the chapter. Probiotics exhibit endless future possibilities for interventions such as the search for a better and improved probiotic dose, dosing strategy or strain combinations, that is why searching for other more randomized controlled trials (RCT) data could be a never-ending job (Zeilstra et al. 2018; Berrington and Zalewski, 2019). The role of *Bifidobacterium* and *Lactobacillus* as the most suitable probiotic agents in the prevention of different infant infections showed the potential efficacy of probiotics to interact with infant gut microbial composition. However, for different infections, specific strains show better results. The mechanism of action of probiotics in the infant is majorly the same as that of adults with the only difference being slow functioning because of the developing immune system. The importance of probiotic infant formula has also been focused majorly to study its viability, side effects, and control on cost. Optimal dosage of administration, most preferred microbe, and species to be used still need further validation. The major threat to the concept of manipulating the intestinal microbiome via other microbes on health is product commercialization of those products that claim health benefits without any sufficient validation. Thus, more studies are required to cover this area.

Along with this, the role of synbiotics—an intervention combining both probiotic and prebiotic components, in infant formula was also discussed. Synbiotics being new to the society is an emerging technique that holds the potential to be employed in the future of serving the pediatric population either in the form of supplements or infant formula. Although there is still much to be learned, probiotics have represented themselves as an efficient part of human life in developing a novel as

well as anti-infectious therapy. Furthermore, upcoming work should be done to address the safety issues related to probiotic-based foods, primarily by enlightening the area of paraprobiotics. Risks associated with antibiotic resistance in preterm infants should also be explored. Lastly, attracting commercial and public interests towards the consumption of probiotics to a bigger level should be also conducted and evaluated.

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Animal Models for Probiotic Interventions Under Gut Inflammatory Conditions

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Abstract

The chapter summarizes the importance of gut microbiota and the relationship between gut microbiota and different inflammatory disease conditions. It also discusses how the commensals and pathogens modulate intestinal barrier functions directly and indirectly, which is the major factor contributing to gut inflammatory disorders. The brain–gut axis involving the hypothalamus–pituitary–adrenal axis and enteric nervous system has a major role in the inflammatory bowel disorder (IBD) pathogenesis. IBD, which is the most common among gut inflammatory disorders, involves a homeostatic imbalance in the microbiota, gut epithelium, and intestinal immune response. This chapter highlights various animal models used to study inflammation related to gut and the management of these pathologies by using different probiotic strains. The probiotics cause immune stimulation through dendritic cells, which further prevents pathogen translocation and strengthens the host immune system. Probiotics, prebiotics, fermentable carbohydrates, and antibiotics are some of the microbiota directed therapies used to modulate host metabolic and immune response in IBD. The most common bacterial strains used in the management of these disorders include *Lactobacillus* and *Bifidobacterium* strains which have

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shown good results in mice model. Understanding the mechanisms involved in disorders related to inflammation in the gut will further help in exploring possible therapies for their management.

Keywords

Probiotics · Gut inflammation · Animal model · Gut microbiota · IBD

Abbreviations

AAD	Antibiotic-Associated Diarrhea
AMP	Antimicrobial Peptides
ANS	Autonomic Nervous System
B-FGF	Basic Fibroblast Growth Factor
CD	Cluster of differentiation
CD	Crohn's Disease
CDAI	Crohn's Disease Activity Index
Cfu	Colony-forming unit
CG	Carrageenan
CLA	Conjugated Linoleic Acid
CNS	Central nervous system
COX 2	Cyclooxygenase-2
DAI	Disease Activity Index
DSS	Dextran Sulfate Sodium
DUOX	Dual Oxidase
ENS	Enteric Nervous System
FAO	Food and Agriculture Organization
FBD	Functional Bowel Disorders
FCA	Freund's Complete Adjuvant
FMT	Fecal Microbiota Transplant
GABA	Gamma aminobutyric acid
GF	Germ-Free
Gn	Gnotobiotic
has	Human Serum Albumin
HPA Axis	Hypothalamus–Pituitary–Adrenal Axis
Hsp	Heat Shock Protein
HSV-1	Herpes Simplex Virus Type 1
IBD	Inflammatory Bowl Disorder
IFN-γ	Interferon gamma
IgG1	Immunoglobulin G1
IL-1	Interleukins
IPAA	Ileal Pouch Anal Anastomosis
ISAPP	International Scientific Association for Probiotics and Prebiotics
ISCs	Intestinal Stem Cells

JAK/STAT	Janus Kinase/Signal Transducer and Activator of Transcription
MAPK	Mitogen-Activated Protein Kinase
MDR	Multiple Drug Resistant
MHC	Major histocompatibility complex
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NF-Kb	Nuclear factor kappa-light-chain-enhancer of activated B cells
NOS	Nitric Oxide Synthase
NPY	Neuropeptide-Y
PBS	Phosphate-buffered saline
PG	Prostaglandins
PML	Progressive Multifocal Leukoencephalopathy
PUFA	Polyunsaturated Fatty Acids
ROS	Reactive Oxygen Species
RT-PCR	Real Time Polymerase Chain Reaction
SCI	Spinal Cord Injury
SCID	Severe combined immunodeficiency
SDF-1	Stromal Cell-Derivedfactor-1
SSCP	Single Strand Conformation Polymorphism
STAT-3	Signal Transducer And Activator Of Transcription 3
TGF-β	Transforming Growth Factor-β
Th 17	T Helper Type 17
TLR	Toll-Like Receptor
TNBS	Trinitrobenzene Sulfonic Acid
TNF-α	Tissue Necrotic Factor-α
Tregs	Regulatory T Cells
UC	Ulcerative Colitis
VEGF	Vascular Endothelial Growth Factor
WHO	World Health Organization

4.1 Introduction

The human digestive tract inhabits approximately 70% of bacterial cells majorly in the colon, i.e. 1000 different types of 10^{14} bacterial cells. The human gut is expected to have a surface area of a tennis court (200 m^2). When a child is born, there is an aseptic environment in his/her alimentary canal, and bacteria begin to occupy it during the birth process (from the maternal vaginal or fecal flora and/or the environment) (Wallace et al. 2011). Initially, facultative anaerobic bacteria (*Escherichia coli* and *Streptococcus* spp.) colonize the large intestine and metabolize any traces of oxygen there and make environment anaerobic. The next bacterial strains to colonize are dependent on the diet of the infant. Breast milk feed for the infants is regarded as a complete food as it contains most of the nutrients, and it also causes alterations in the probiotic levels of infant gut (Gibson and Roberfroid 1995). Bacterial colonies

present in infant's gut are determined by microbiota and hygiene of the mother's genital tract, mode of delivery (vaginal or cesarean), and breast or bottle feeding. The final microbial colonies are acquired at weaning when a plethora of microflora develops (Fuller 2012). Non-sporing anaerobes such as *Lactobacillus* spp., *Fusobacterium* spp., *Bacteroides* spp. and *Bifidobacterium* spp., *Clostridium* spp., *Eubacterium* spp., and many gram-positive cocci constitute the major proportion of gut bacteria (Macfarlane 1991). *Enterobacteriaceae*, *Enterococcus* spp., methanogens, and some sulfate-reducing bacteria are present in a very low amount in the adult gut (Sekirov et al. 2010; Wallace et al. 2011).

The whole bacterial community that exists peacefully with the host when taken together is referred to as gut microbiota, or normal flora or microbiome (Lakshminarayanan et al. 2014). It has already been proven that microbiota influences several important functions of the host's physiology and metabolism. During early development, it helps in the development of the immune response and contributes to the process of life-long homeostasis. Healthy and natural gut microbiota plays a key role and is an essential factor that controls metabolism, prevents autoimmunity, protests from diseases, and provides resistance in conditions such as infection, inflammation, and cancer. The gut microbiota also plays an important role in regulating the brain–gut axis (Vaiserman et al. 2017; Konturek et al. 2015). Besides, it is shown that the gut microbiota can impact the risk of gastrointestinal pathologies such as irritable bowel syndrome, inflammatory bowel disease, colorectal cancer, and also few other disorders, which include the ones that influence the respiratory tract (allergy, bronchial asthma, cystic fibrosis, etc.) and the liver. The most common characteristic of these diseases is the change in the number and composition of the typical microbial population of a healthy human intestinal tract that includes species such as populations of the *Bifidobacterium* genus. Probiotics that contain *Bifidobacterium* spp. offer prevailing and probable solutions to treat these and other different diseases (Arboleya et al. 2016; Vaiserman et al. 2017).

The age-related changes include a decline in microbiota diversity, changes in the gut microbiota composition, an expansion in proteolytic bacteria and reduced saccharolytic bacteria, increased quantity of subdominant species, and diminished wealth of core (dominant) species. It also shows increased *Proteobacteria*, reduced bifidobacterial counts, and a lowered ratio of *Firmicutes* to *Bacteroides* (F/B) (Biagi et al. 2016; Bischoff and Care 2016; Konturek et al. 2015; Perez Martinez et al. 2014; Vaiserman et al. 2017). Summary of age-related changes at the genus and species level in the relative composition of human microbial composition is shown in Table 4.1. The changes can lead to various known age-associated pathological states, such as neurodegeneration, chronic inflammation, frailty, diabetes (type 1 and type 2), cognitive decline, as well as cardiovascular disease and non-alcoholic fatty liver disease (Vaiserman et al. 2017). The microbiota is additionally perceived as a key factor that can modulate the risk of cancer development, probably utilizing the activity of the specific factors, such as microbial structures, metabolites, particular toxins, and modulated immune responses (Pope et al. 2017).

Table 4.1 Summarized findings of age-related changes at the genus and species level in the relative composition of the human microbial composition (Vaiserman et al. 2017)

Taxon	Age-related trends	
	Decreases	Increases
1. Minor Groups		
• Clostridium abundance		✓
• Bacteroides species diversity		✓
• Providencia and Proteus species		✓
• Bifidobacterial species diversity	✓	
• Bifidobacteria	✓	
• Enterobacter and Klebsiella species	✓	
• Faecalibacterium prausnitzii	✓	
• Facultative anaerobes (streptococci, staphylococci, enterococci, enterobacteria)	✓	
2. Phyla		
• Firmicutes	✓	
		✓
• Bacteroidetes	✓	
		✓
• F/B ratio	✓	

4.2 Gut Microbiota

Joshua Lederberg initially introduced the term "gut microbiota" to the researchers, who defined it as "the ecological community of commensal, symbiotic, and pathogenic microorganism that share our body space and have been all but ignored as determinants of health and diseases"(Lakshminarayanan et al. 2014). The humans comprise of trillions of microbes which are mostly present in the GI tract within the small intestine and colon) (Azad et al. 2018; Sender et al. 2016). The gut microbiota is capable of fermenting non-digestible carbohydrates, which are commonly known as prebiotics, which include inulin, oligofructose, fructo-oligosaccharide, xylose, and galactose. These prebiotics contain oligosaccharides which satisfy complete energy requirements. The microbes that are present in the host body affect physiology, metabolism, and immune development and significantly perform a different function. The symbiotic function of these microbes includes vitamin synthesis, protection against pathogenic colonization, GI hormone release modulation for immune system regulation, and also brain-behavior regulation via neuronal signaling (Azad et al. 2018; Bin et al. 2017; LeBlanc et al. 2013; Martel-Pelletier et al. 2001; Round and Mazmanian 2009).

The human gut exists as a multiplex ecosystem which consists of host cells, the microbiota, and nutrients interconnected extensively. These connections have long been thought out only from a pathogenic perspective considering that toxins attack the gut mucosa and transport, spread, and result in systemic infections (Butel 2014).

However, no consideration was paid to the association between host health and most of the gut microorganisms. Several reports reveal valuable interactions between the human body and commensal microbiota and also reported that the microbiota plays the role of a genuine partner. A more insightful understanding of the role of gut microbiota in human health is necessary for prospective healthcare strategies. In such a manner, a detailed exploration of the possible utilization of different strains of probiotic bacteria is frantically required for the prevention and treatment of various ailments related to humans and animals. The fact that gut microbiota has a major role in affecting the health of the host has attracted usage of probiotics to prevent gut-related diseases in human and animals (Kitazawa et al. 2013; Bron et al. 2012; Lebeer et al. 2010; Kitazawa et al. 2015).

The advancement in molecular high-throughput techniques supports the identification of bacteria that were not known in the past and this provides a novel understanding of the compositional diversity and functional capacity of some of the fecal microbiota. Additionally, several reports suggest that disorders, viz., inflammatory bowel disease (IBD), colorectal cancer, fatty liver diseases, oxidative stress-related disease, type 2 diabetes, immunological diseases, and obesity, are associated with dibiotic modified microbiota compositions which are disease-specific (Feng et al. 2015; Nobili et al. 2018; Park et al. 2018). Alteration of the gut microbiota has therefore been considered as one of the prospective treatment options for multiple diseases in both humans and in animals (Azad et al. 2018; Feng et al. 2015; Li et al. 2016; Nobili et al. 2018; Park et al. 2018).

4.3 Prebiotics and Probiotics

The term prebiotic is defined as a non-viable food substance that provides a health benefit to host by modulation of the intestinal microbiota and promotes the selective growth of beneficial bacteria. Probiotics are termed as live microorganisms; administration of probiotics in sufficient amounts provides health benefit to receiving host organisms. Probiotics play an important in regulating the host immune system and also maintaining the gut barrier function. The use of dietary supplements that contain probiotics can be a beneficial and useful option for IBD. *Lactobacillus*, *Enterococcus,* and *Bifidobacterium* strains are normally utilized as probiotics (Park et al. 2018).

Modifying gut microbiota with the use of probiotics is a regular approach to treat human and animal diseases. More attention has now been diverted to study the production of probiotic food and its application in alteration of the gut microbiota. Since the beginning of the twentieth century, probiotic foods containing a mix of bacteria has been given importance and their supplement and consumption has been studied widely, and yogurt has particularly drawn attention in maintaining great well-being using its role in the digestive system and prevention of different degenerative ailments (La Fata et al. 2018; Brown and Valiere 2004).

The word “probiotic” originated in Greek and the word signifies “for life.” In 1954, an article entitled “Anti-und Probiotika,” by Ferdinand Vergin considered the

term "probiotic" where different microorganisms were considered to create a list of important bacteria and to find out effects of antibiotics and antibacterial compounds on the gut microbiota (Azad et al. 2018; Vergin 1954). Lilly and Stillwell after few years described probiotics as beneficial microbes promoting growth factors for other microorganisms (Lilly and Stillwell 1965). The term "probiotics" has undergone modifications over a period of time and several studies are being conducted looking into its diverse applications which include clinical trials for several human and animal models. The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) defined probiotics as live microorganisms that provide different health benefits to the host when administrated in sufficient amounts. This definition by WHO and FAO is trailed by the International Scientific Association for Probiotics and Prebiotics (ISAPP) (Food and bulletin 2008; Hill et al. 2014). Probiotics are generally regarded as safe for human health and have long been considered for human health improvement. The researchers are continuously developing new probiotics with improved applications. The majority of probiotics available nowadays are mainly created with *Lactobacilli, Bifidobacteria*, and several other lactic acid bacteria, viz., *Streptococci and Lactococci.* New potential probiotic strains that are considered are of bacterial genera *Escherichia, Propionibacterium* and *Bacillus*, and few of the yeast genera, mostly *Saccharomyces* (He and Shi 2017). Several species and strains of *Lactobacilli* have been broadly considered for development of probiotics and that includes *Lactobacillus casei, Lactobacillus helveticus, Lactobacillus acidophilus,* and *Lactobacillus rhamnosus*. The probiotic species are known to modify the number of microorganisms in the gut microbiota and can control the working of the overall ecosystem of gut microbiota. Several investigations have reported credible evidences of clinical trials which show probiotics use in animal and human models and its application in the treatment of different diseases, and these studies have been increasing considerably (Jiminez et al. 2015; Butel 2014).

4.4 Relationship Between Gut Microbiota and Disease Condition

Gut microbiota is known to be an extremely important factor that drives the inflammation. Despite the evidence that microorganisms are essential in driving inflammation, some microbial species of different genera (*Bifidobacterium, Lactobacillus*, and fecal bacterium) are known to defend the mucosa from inapt inflammatory responses that might harm the host. Some strains of bacteria (*Lactobacillus plantarum, Lactobacillus casei*, and *Faecalibacterium prausnitzii*) neutralize the proinflammatory effects of *E. coli* by downregulating the expression of key proinflammatory cytokines and chemokines of *E. coli*. In addition, a few strains of the previously mentioned genera can trigger anti-inflammatory cytokine IL-10 production. Therefore, certain members of the gut microbial community may aggravate inflammation, however, some other members can alleviate inflammatory reactions by inducing immune-regulatory pathways (Manichanh et al. 2012).

The commensals are involved in the regulation of pathogens by direct or indirect means. Symbionts refer to some of the commensals that are useful to the host, whereas pathobionts are dangerous to the host under certain conditions (Hornef 2015). The commensal microbiota controls the colony of both invading pathogens and pathobionts directly and indirectly. For example, symbionts cause the release of bacteriocins and proteinaceous toxins which directly can target or kill similar bacterial strains (Hammami et al. 2013). Likewise, metabolically related pathogens and symbionts compete for scarce nutrients, this allows symbionts to oppose colonization of associated pathogens in the gut (Kamada et al. 2012).

The by-products of commensal bacteria indirectly oppose the colonization of pathogens (Bjursell et al. 2006). Microbiota can also indirectly stimulate the host immune system and exert protective effects on pathogens. The stimulation of epithelium by the microbiota causes the production of antimicrobial peptides and mucus, which are important in maintaining critical sterile mucus inner layer against both pathogens and commensals (Jakobsson et al. 2015). Several antimicrobial peptides and proteins are expressed by microbiota, e.g. constitutively expressed α-defensins are expressed without any bacterial signals, whereas *B. thetaiotaomicron* (Gram-negative bacteria) induce the expression antimicrobial peptides RegIIIγ which shows explicit microbicidal activity against Gram-positive bacteria. The pathogens tend to attach to the intestinal epithelium surface at the earliest enteric infection stage and this attachment is prevented by commensals through direct or indirect regulation of pathogens (Pütsep et al. 2000; Cash et al. 2006).

Investigational evidence in animal models shows that several commensal bacteria of the dominant gut microbiota play a key role in maintaining inflammation of intestinal mucosal within "physiological" levels. This prevents epithelial cell damage by different mechanisms, which includes an expansion of TREG cell subsets or direct suppression of inflammatory pathways. Some bacteria even inhibit the activation of NF-κβ and it may lead to a decrease in the production of proinflammatory cytokines (Manichanh et al. 2012). The association between the commensal flora and the immune system is treacherous, and change in this relationship may lead to a gut inflammatory condition. Several studies have reported that, in the case of inflammatory bowel disease (IBD), bacterial flora contrast between healthy people and people suffering from IBD. In IBD patients, grave consequences can be observed if the improper response towards resident bacteria is not controlled in time. Furthermore, in the ileal mucosa of patients with Crohn's disease, marked adherent or invasive strains of *Escherichia coli* have been recognized and the involvement of a new potentially pathogenic group of adherent invasive *E. coli* has been proposed. Lately, the collapsed balance between putative species of "protective" versus "harmful" intestinal bacteria has been hypothesized and the concept is referred to as "dysbiosis" (Conte et al. 2006).

The link between microbiota and immune response of the body has largely been explored. Different bacterial species are linked to different pathologies, for example, an increased number of *E. coli* are known to cause the pathological condition as dysbiosis whereas decreased diversity of *Firmicutes* and *Bacteroidetes* can lead to

probiosis which is a disease protective effect. Elie Metchnikoff won the Nobel Prize in 1908 for studying the involvement of nutrition and gut microbiota in human well-being. He gave the idea that our colon is full of intoxicating bacteria that are killed by eating milk products that can promote the growth of healthy bacteria, for example, *Lactic bacilli* in curd can help our body fight these intoxicating bacteria. Imbalanced gut microbiome is also linked to various other disorders, for example, inflammatory bowel disease, allergy, cancer, diabetes, obesity, cardiovascular abnormalities, irritable bowel syndrome, neuro-pathologies (Kaufmann 2008; Holmes et al. 2011).

Various models for studying the involvement of gut flora in the immune system have already been used. One of the tools is to conduct a study with the use of Germ-free or gnotobiotic animals colonized with desired bacteria to provide an idea that microbiota has a pivotal role in the maintenance and advancement of the immune system of the host. Germ-free animals showed defects in gut lymphoid tissue development of which was retracted by exposing them to Gram-negative bacteria indicating a relationship between microbiota and development of lymphoid tissues. Regulatory T cells (Tregs) which are reduced in GF mice also get restored after colonization of healthy microbiota, for example, *Bacteroides fragilis* or its product, polysaccharide A (Hooper et al. 2012).

4.5 Mechanisms Involved in IBD

The primary features of any autoimmune and inflammatory diseases are tissue damage and functional injury of a particular organ. Fig. 4.1 summarizes the mechanisms involved in IBD and potential targets for treatment. This can be induced in principle by immunologically driven mechanisms that are similar to the ones that might function against pathogens. Living bacteria, their metabolites, and their components are noticeably accountable for major immunomodulatory mechanisms. Substantial investigations on inflammatory, neoplastic, and autoimmune diseases are focused on determining the pathogenic role of various microbial components. In the case of functional impairment of the intestinal barrier, it has been observed that impairment prompts an expansion in antibodies against the antigens present in the intestinal lumen. Recently, it was observed that the presence of these antibodies in individuals without clinical symptoms might play a vital role in predicting the development of autoimmune and inflammatory diseases (Tlaskalova-Hogenova et al. 2011).

IBD is a term used to describe the chronic inflammation associated with the digestive tract. Types of IBD include Crohn's disease (CD) and ulcerative colitis (UC). The pathogenesis for both CD and UC is not understood. The studies suggest that the chronic inflammation might be resulting from the atypical and dysregulated immune response to the intestinal microbiota and to some extent it might also be related to the genetic susceptibility. In the case of IBD, the decreased immune tolerance directed at the enteric flora is intervened by various molecules, for example, cytokines like interleukins, TNF-α, TGF-β. Along with conventionally known

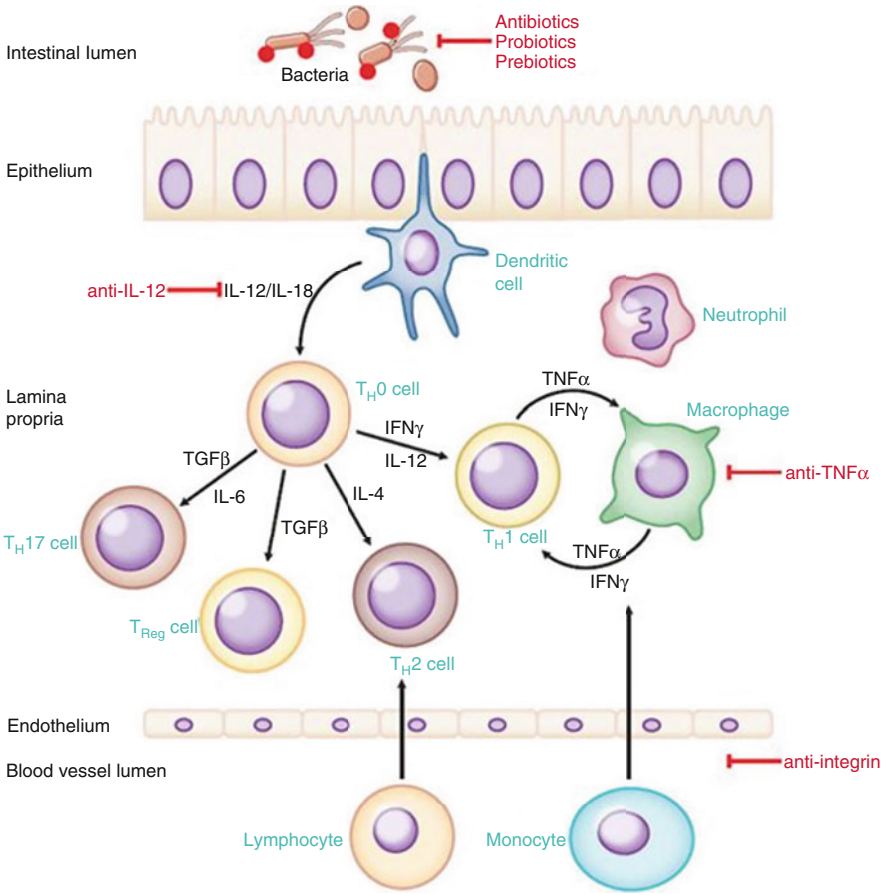

Fig. 4.1 Mechanism involved in IBD and the potential targets for the treatment. Antibiotics, probiotics, and prebiotics can act as bacterial antigens and interact with immune cells in the intestine. Epithelial barrier disruption gives access to these antigens which can then interact with antigen-presenting cells (APC), for example, dendritic cells present in lamina propria of intestine. These cells present antigens to $CD4^+$cells and secrete proinflammatory cytokines such as interleukins (IL-12, IL-18, and IL-4) in the case of CD and UC. The balance between proinflammatory and inflammatory events is managed by helper T(Th) cells and regulatory T (Treg) cells. TGF-β and IL-6 control the expansion of Treg cells whereas Th_1 produces TNF-α and IFN-γ which then activate macrophages. Macrophages in turn activate Th1 to produce TNF-α and IFN-γ. Cell adhesion molecules such as integrins are involved in the infiltration of leukocytes which are involved in the inflammation (Goodman 1996)

interleukins, viz., IL- 5, IL-6, IL-4, IL-1, novel interleukins such as IL-21, IL-22 are involved in IBD (Sanchez-Munoz et al. 2008).

UC, idiopathic IBD, and Crohn's disease affect nearly 0.2% of western population. In the pathogenesis of IBD, the interactions between environmental, genetic, and immune factors are involved and these interactions induce the inflammation

leading to subsequent mucosal lesions development and repair. In the pathogenesis of chronic intestinal inflammation disrupted T lymphocyte regulatory functions and impaired mucosal immune response to normal bacterial flora play an important role. This may be perhaps associated with the breakdown of oral tolerance to luminal antigens and loss of local physiological regulatory mechanisms in these diseases (Singh et al. 2001; Uhlig et al. 2006). In human IBD, T-cells respond abnormally against indigenous microbiota and this suggests that commensals may initiate and/or maybe responsible for the intestinal inflammation observed in IBD. The symptoms involved in IBD constitute gastrointestinal motility changes and visceral hypersensitivity. Decreased or increased motility can be treated by pharmacological agents used to treat constipation or diarrhea. Increased sensitivity to stimuli is also a common symptom in IBD which occurs due to increased perception of receptions in the gut wall which sends signals to the dorsal horn of the spinal cord via afferent neural pathways and finally to the brain.

Microbiota composition changes in various inflammatory diseases have been studied. A bacterium that was found to be decreased in CD is *Faecalibacterium prausnitzii* belonging to phylum Firmicutes while *Escherichia coli* belonging to phylum Proteobacteria was increased in IBD patients leading to dysbiosis (Ni et al. 2017).

The role of various growth factors in inflammatory gut diseases mainly UC and CD has been studied. Serum levels of vascular endothelial growth factor (VEGF) were found to be increased in both UC and CD patients but not in healthy individuals. VEGF is released from inflamed tissues in the case of these patients. The mucosal expression of TGF-β was also found to be increased in both CD and UC patients. Increased serum basic fibroblast growth factor (b-FGF) and VEGF levels were observed in various skin diseases involving collagen suggesting their antagonist might contribute to regulating the immune system (Kanazawa et al. 2001). Diet also plays a key role in the development of inflammatory diseases, for example, dietary n-6 polyunsaturated fatty acids (PUFA) and reduced consumption of marine n-3 PUFA are inversely related to the occurrence of inflammatory diseases. On the other hand, supplementation of serum vitamin D, vitamin A, higher dietary zinc, and polyphenols, viz., resveratrol and flavone has shown to decrease the occurrence of IBD (Ananthakrishnan and 360 2020; Barbalho et al. 2019).

In a review by Silvio Danese in 2011, various new mechanisms for IBD have been mentioned including the cytokines (TNF-α, IL-12, IL-6, IL-23), the interaction between the gut microbiota and the host leading to dysbiosis, the role of defensins as an antimicrobial peptide in immunodeficiency which is a main player of inflammation and the equilibrium between coagulation and inflammation (Danese 2011). Chemokines and Stromal cell-derived factor-1 (SDF-1) have been found to play a key role in the pathogenesis of colorectal cancer. Chemokines are cytokines with chemoattractant properties that assist in many cellular functions whereas Stromal cell-derived factor-1 (SDF-1) is expressed in stromal cells and interacts with the seven-transmembrane, G protein-coupled receptor CXCR4. In human colon cancer HT29 cell line, the viability and migration of cells were increased after treating cells

with SDF-1. Immunochemistry showed the role of SDF-1 in downregulating the activation of CXCR4 expression at protein and mRNA levels (Wang et al. 2011).

The neuroendocrine peptides/amines also known as neuropeptides also have shown a role in modulating the immune system. DSS induced colitis in mice caused an increase in neuropeptide-Y (NPY) enteric neurons and hyperplasia of NPY nerve fibers. Neuropeptides have a more role in regulating stress, thus it is expected to have some role in IBD due to the involvement of the gut–brain axis. Activation of the hypothalamus by the cytokines (interleukins, TNF-α) during inflammation is also confirmed by determining levels of neuropeptides. NPY antagonists can be explored as therapeutic agents to treat IBD (El-Salhy and Hausken 2016).

The role of various genes in the pathogenesis of IBD has been studied by single nucleotide polymorphism, knockout, and transgenic models. Various synthesis inhibitors have been explored in the clinics as discussed below:

Interleukin Inhibitors The transcription levels of Interleukin, IL-2 were found to be more in the intestinal mucosa of patients suffering from Crohn's disease (CD) but not in the ulcerative colitis patients (Mullin et al. 1992). Interleukin (IL-10) administration in enema leads to the downregulation of releases of TNF-α and IL-1β in intestinal lamina propria mononuclear cells (LPMNC) and peripheral monocytes of blood in patients suffering from IBD (Schreiber et al. 1995). Interleukin, IL-17 which is a glycoprotein of molecular weight ∼20 kDa with 155 amino acids was found to be over expressed in the intestinal mucosa of patients of IBD (Fujino et al. 2003).

Anti-TNF-α Mucosal inflammation in Crohn's disease was found to be related to increased expression of TNF-α produced by T helper 1 subclass of T cells. Anti-TNF-α (corticosteroids, azathioprine, or mercaptopurine) used against patients of CD showed reduced inflammation. An open-label trial of chimeric monoclonal antibody cA2 as a single infusion was effective against CD (Targan et al. 1997).

Immunomodulators In a Crohn's Trial of the Fully Human Antibody Adalimumab for Remission Maintenance (CHARM) which was a randomized double-blind phase trial on 160 patients, adalimumab, which is an immunoglobulin G1 (IgG1) monoclonal antibody, has shown its effect in maintaining long-term (56-week) remission in CD patients (Colombel et al. 2007). Natalizumab, selective adhesion molecule inhibitors, is a recombinant humanized IgG4 monoclonal antibody (MacDonald and McDonald 2007).

Anti α4 Integrin Natalizumab has been linked to the development of progressive multifocal leukoencephalopathy (PML) which is a rare and often fatal viral disease that causes inflammation of the white matter in the brain (MacDonald and McDonald 2007; Nelson et al. 2018). Natalizumab and Vedolizumab (also an anti-α4 integrin drug) are effective for inducing remission and response in patients with Crohn's disease, with similar efficacy in anti-TNF-α exposed and naive patients (Park and Jeen 2018).

4.6 Inflammatory Conditions Related to Gut

The most common inflammatory conditions related to the gut are discussed below:

Ulcerative colitis (UC): DDS-induced murine model is the most common model to study UC (Kiesler et al. 2015). Colon epithelium of UC patients showed more lipid peroxidation and ROS formation in mitochondria with abnormal bioenergetics. Also, ultramicroscopy of the colon showed abnormal mitochondria in the mucosal region (Santhanam et al. 2012). DSS-induced colitis model showed symptoms like human UC which includes progression in the inflammatory response, clinical signs (colon length shortening, diarrhea, bloody stools), histopathological changes in colon, and alterations in gut microbiota (Chassaing et al. 2014). Polyphenols present in apple peel have shown a protective effect against DSS-induced colitis owing to its anti-inflammatory and antioxidant properties (Denis et al. 2016; Yeganeh et al. 2018). A study by Park et al. showed the effectiveness of the combination of probiotics, prebiotics, rosavin, and zinc against DSS-induced colitis (Fig. 4.2) (Park et al. 2018).

Crohn's disease is associated with immunological stigmata which show a highly increased CD4 T helper cell type I response. Therefore, in case of Crohn's patients the isolated intestinal CD4 T cells produce increased quantity of the Th1 signature cytokine interferon-γ and it also displays striking overexpression of T-bet (Th1 cell-specific transcription factor). It was also observed that mucosal macrophages of Crohn's patients also produce a great amount of the Th1-inducing cytokines, IL-18 and IL-12. In Crohn's disease inflammation, the Th1 cell resistance to apoptosis and increased cell cycling appears to be sustained by cytokines. Attempts which are successful in down-modulating intestinal inflammation are obstruction of the pathways which leads to the resistance of Th1 cells to apoptotic stimuli and the use of drugs that increase mucosal T-cell death, such as antibody to TNF-α infliximab or the immunosuppressive agent azathioprine (Tlaskalova-Hogenova et al. 2011).

Intestinal mucositis is an inflammation of the mucosal layer of the intestine and affects the entire gastrointestinal system. It is a common side-effect related to chemotherapy and caused bacteremia and malnutrition in the patients which requires hospitalizations. It is linked with activation of the NF-κB pathway which further activates proinflammatory markers such as cytokines including interleukins and Tissue Necrotic Factor-α (TNF-α). A probiotic mixture of strains *Lactobacillus* and *Bifidobacterium* attenuated complications associated with 5-Fluorouracil induced intestinal mucositis model. Also, it reduced cytokine levels (IL-6 and TNF-α) and normalized gastric motility (Quaresma et al. 2019). In addition to this, bacterial strain *S. boulardii* CNCM I-745 (107 CFU/mouse) used alone also decreased inflammation and restored abnormal motility of GI tract (Brun et al. 2017). Oral administration of suspension made up of probiotic *Lactobacillus casei* variety *rhamnosus* in Balb/c mice caused a reduction in the expression of proinflammatory cytokines IL-1β, TNF-α, and downregulations of the lymphocytes CD3+/CD8+ and CD8+/IFN-γ cells suggesting an anti-inflammatory effect of probiotics (Yeung et al. 2020).

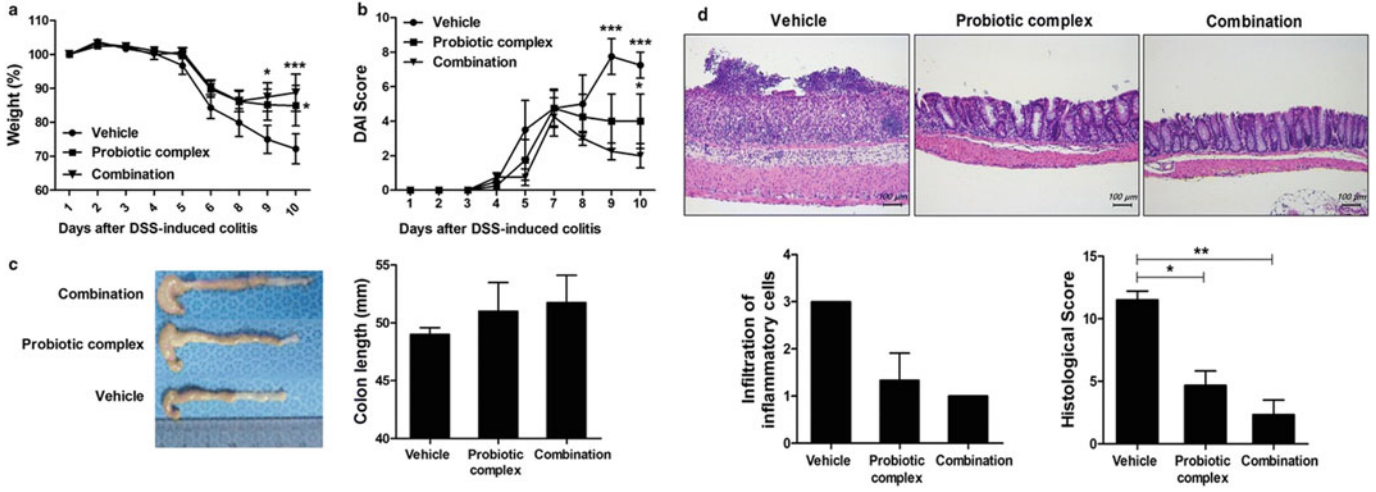

Fig. 4.2 (**a–d**): DSS-induced colitis which was reduced by using a combination of probiotics, prebiotics, and rosavin. (**a**) Body weight variation expressed as a percentage on day 0. (**b**) DAI was monitored daily except 7th day. Data are means ± SDs of four experimental repeats. (**c**) Colon length of mice with DSS-induced chronic colitis on day 10. (**d**) Representative images of colon stained by hematoxylin and eosin on the 10th day (Park et al. 2018)

Intestinal fibrosis is the major pathologic condition in patients who suffer from chronic inflammatory gut diseases. It can then lead to further complications such as epilepsy, lung fibrosis, kidney, and heart disease by increasing the accumulation of connective tissues in various organs. Transforming growth factor-β (TGF-β) has a known important part in cellular proliferation, differentiation, and development and it is also the master regulator that drives fibrosis in all organs. Also, it regulates embryonic development, immunity, carcinogenesis, inflammation, and fibrosis. In normal conditions, its signaling maintains tissue homeostasis via the regulation of cell proliferation while in abnormal conditions it speeds up the progression of cancer and fibrosis. Therefore, inhibitors of TGF-β would be explored to treat fibrosis related to chronic IBD (Yun et al. 2019).

4.7 Gut–Brain Microbiome Axis and IBD

Recently, the interaction between gut and brain has come into the focus of researchers to find its role in IBD. Brains' ability to alter intestinal functions is well known but the gut effect on mood and cognitive functions is less explored. Our digestive system is very complex with a larger surface area for absorption of about 300 square meters and a highly complex hormonal system comprising of more than thirty hormones. It is highly innervated by the enteric nervous system which controls bowel movements (Furness 2012). This communication involves the participation of different members of the gut–brain microbiome axis, viz., gut microbiota; ENS, autonomic nervous system (ANS), CNS. An alteration in gut microbiota leads to stress. The functions of the intestine are controlled by ENS when it is completely separated from CNS. The ENS comprises three types of neurons: motor, associative, and sensory neurons that can be both inhibitory and excitatory which control signals to the musculature, both blood and lymphatic, mucosal layer and microbiota's homeostasis. It contains a large number of neurons (400–600 million) approximately number of neurons presnt in the spinal cord and provides autonomy to the gastric tract for motility and various secretions. In ANS, the afferent fibers that enter the CNS from the gut are invalid in sending sensory, chemical, mechanical, nociceptive, thermic signals. Modulation of ENS is also done by efferent fibers present in the parasympathetic (vagus and pelvic nerves) and the sympathetic (the splanchnic nerves) branches of ANS that lead to the release of noradrenaline and acetylcholine (Pellissier and Bonaz 2017). The different brain signals sent to gastrointestinal tract by hypothalamic-pituitary-adrenal (HPA) axis and sympatho-adrenal axis, causes modifications in spinal reflexes and dorsal horn excitability. Gut to brain bidirectional communication involves neuronal, endocrinal, and immune afferent signaling (Al Omran and Aziz 2014).

In one study, dextran sulfate sodium (DSS)-induced colitis has shown alteration in neuropeptide expression and stress-related behavior in mice model. In water avoidance stress, mice showed prolonged immobility which can be correlated with altered proinflammatory cytokines (ILs) and neuropeptides expression in brain and circulating corticosterone levels. Psychological stress increased colonic severity

which suggests the role of stress in colitis and further indicating altered HPA axis in colitis. Mice with experimentally induced colitis showed less grooming which is common in depression where self-care and motivation are absent (Reichmann et al. 2015). In healthy human volunteers, there was found a relationship between gut microbiota and mood. Psychobiotics are the probiotics which are known to have mood-enhancement properties. In one study, psychobiotics (*Lactobacillus spp.* and *Bifidobacterium* spp.) have shown to reduce anxiety among participants (Taylor et al. 2019). A study by Messaoudi et al. has also shown that probiotic combination of *Lactobacillus helveticus* and *Bifidobacterium longum* attenuated stress-like behavior in rats and treated psychological stress and improved sleep quality in human volunteers. These results also proved gut microbiota has an important role in stress, anxiety, and depression via ENS (Messaoudi et al. 2011). Bravo, Javier A. et al. showed *Lactobacillus rhamnosus* caused region-dependent changes in $GABA_{B1b}$ receptor mRNA expression in the brain of mice with more concentration (GABA) in cortical regions (cingulate and prelimbic) and reductions in the hippocampus, amygdala, and locus coeruleus (Bravo et al. 2011). *L. rhamnosus* also reduced stress, anxiety, and depression-related behavior. In human colon epithelial cell lines, *Lactobacillus* has shown decreased levels of proinflammatory cytokines; IL-8 and TNF-α (Ko et al. 2007; Bravo et al. 2011; Ko et al. 2007). A double-blind, randomized study performed on healthy volunteers proved the positive effect of probiotics formulation (*Bifidobacterium spp.*, *Lactobacillus spp.)* on mood, e.g. depression and anxiety-like symptoms as compared to the volunteers who were given placebo formulation (Bagga et al. 2018). Another longitudinal double-blind randomized design study showed 4 weeks of probiotic supplementation affected cognition positively (Papalini et al. 2019). In another study, a combination of probiotics *(Lactobacillus helveticus*, *Lactobacillus plantarum*, and *Bifidobacterium longum*) was used for treating depression in mice with chronic mild stress (CMS). Four weeks of treatment with this probiotic formulation by oral gavage to mice reduced anxiety-like behavioral changes and proinflammatory cytokines (TNF-α, IFN-γ andIL-6) in the hippocampus (Li et al. 2018). A structural diagnostic interview in the nationals of America and New Zealand who were suffering from IBD showed more prevalence of anxiety and mood-related disorders (Walker et al. 2008).

4.8 Treatments Available

There are no treatment options available as such for IBD; mostly symptoms are treated with the help of anti-inflammatory drugs and surgeries. Drugs used clinically include corticosteroids, 5-aminosalicylic acid compounds, immunomodulators such as azathioprine plus mercaptopurine, infliximab, methotrexate, antibacterial, thalidomide by inhibiting the synthesis of cytokines and defined formula diets mostly used in toddlers. All the treatments are associated with various side-effects that could be mild such as nausea, anorexia, vomiting, arthralgia, skin rash, or severe such as pancreatitis, opportunistic infections, bone marrow suppression, non-Hodgkin's

lymphoma, and hepatotoxicity, therefore, cautious monitoring by medical professionals is required while undergoing any treatment (Lanzarotto et al. 2006; Fakhoury et al. 2014). Protective bacterial strains have been used as an adjunct therapy to immunosuppressive agents in managing IBD.

Probiotics have been considered as a prospective therapeutic strategy for IBD patients, noticeably for those who are seeking "natural and safe" approaches. Several clinical interventional studies reported the positive outcomes obtained with various animal models of colitis and have revealed the beneficial effects of some probiotics in patients suffering from UC or pouchitis (Ghouri et al. 2014). These protective effects of probiotics were strain-dependent, and various well-characterized probiotic strains when tested failed to satisfy the expected clinical outcome, particularly in the case of patients with Crohn's disease (Manichanh et al. 2006). In another study of trinitrobenzene sulfonic acid (TNBS)-induced colitis in mice, the protective effect of *lactobacilli* and *bifidobacteria* against TNBS was reported to be strain-specific and is well correlated with *in vitro* immunomodulatory characteristics of the species that were reported in a previous study. The strains that showed best results in *in vivo* colitis model were showing induction of higher levels ofIL-10 and reduced levels of IFN-γ, IL-12, and pro-Th1 cytokines after *in vitro* activation of human peripheral mononuclear cells (PBMC). On the other hand, strains that were inducing a low IL-10/IL-12 cytokine ratio inhuman PBMCs could not notably reduce the colitis symptoms in mice (Foligne et al. 2007).

Commensal microbes are known to restore dysbiosis which is unbalanced gut microbiota and to promote the immune system. Fecal microbiota transplant (FMT) has recently proved to be effective in recurrent or refractory *Clostridium difficile* infection (rCDI). Probiotic strains belonging to genera, *Bifidobacterium* and *Lactobacillus* have shown good results in IBD trials. Recently, bacterial colonies isolated from the microbiota of healthy humans including *Clostridium*, *Firmicutes* spores, *Bacteroides*, *Roseburia, etc.* have been used in clinical trials. Prebiotics including dietary fiber and oligosaccharides which can be found naturally in fruits, vegetables, and grains have shown increased healthy bacterial colonies in the gut of both murine models and humans (Oka and Sartor 2020).

4.9 Preclinical Models Used to Study IBD

A preclinical model used to study IBD mainly includes mice and rats. Other animals used to study IBD are pig, guinea pig, nematode, drosophila, etc. In the murine model, IBD can be used spontaneously because of the presence of genes to aggravate immune response; chemicals can damage intestinal mucosa directly and cause inflammation or genetically modified mice. Some of the widely used models are described in the following sections:

4.9.1 Spontaneous Models

Spontaneous models of colitis can be induced in individuals with an increase in the genetic susceptibility with alterations in environmental factors.

C3H/HeJBir A substrain of C3H/HeJ mice develops colitis simultaneously. Inflammation was not present in any organ other than the cecum and right colon. There have been many previous reports showing the presence of various susceptibility genes responsible for the induction of chronic intestinal inflammation (Mähler et al. 1998). CD41 T cells isolated from C3H/HeJBir were strongly reactive to bacterial flora antigens present in the intestine but not to epithelial or food antigens indicating the T cell reactivity of C3H/HeJBir mice to these antigens that might be involved in the pathology of disease (Cong et al. 1998). C3H/HeJBir showed more susceptibility to DSS-induced inflammation in both colon and cecum (Mähler et al. 1998).

Cotton-top Tamarin Colitis Spontaneous induction of chronic colitis in primate, cotton-top tamarin (*Saguinus oedipus*) makes it as an ideal model for studying the genetic basis for the adenocarcinoma in colitis. These monkeys have only one major histocompatibility complex (MHC) class I locus which along with the environmental factors contributes to the spontaneous models of colitis (Watkins et al. 1990; Madara et al. 1985). Antibodies against TNF-α and α4-integrin showed effectiveness against colitis in CTT proving the role of proinflammatory cytokines and leukocyte vascular adhesion in the inflammation related to colitis (Watkins et al. 1997; Podolsky et al. 1993).

4.9.2 Chemical Model: *Inducible Colitis Models*

Chemicals and mechanical agents can be used to induce colitis by causing inflammation in the mucosal barrier.

Formalin/Immune Complexes Induced Colitis 1% formalin (1 mL) given rectally to young New Zealand white rabbits induced acute colitis which was changed to mimic chronic colitis by giving a prior subcutaneous injection of bacterial antigen along with injections of antigen-antibody complexes like human serum albumin (HSA) and anti-HSA made in antigen excess. The mechanism for this is stated as initially acute colitis is induced by immune complexes which were deposited in formalin-damaged mucosa made the absorption of bacterial antigens easy into the colonic mucosa. In the immunized animals, this could cause local antigen–antibody interactions and thereafter tissue damage (Mee et al. 1979).

Acetic Acid-Induced Colitis Trans-rectal treatment of 4% acetic acid to mice causes mucosal damage and epithelial injury in the colon. It is considered as applied and easily inducible model which offers close similarity with Human IBD in terms of

pathophysiology, histology, and inflammatory mediators' production. Obestatin which is a 23-amino acid peptide derived from the proghrelin and Camellia Oil obtained from *Camellia brevistyla* has shown anti-inflammatory activity against this model (Lee et al. 2018; Matuszyk et al. 2016).

Carrageenan Induced Colitis Carrageenan is a polysaccharide extracted from marine red algae. It is used as a food additive for its good gelation and thickening properties. To induce mild ulcers, 1% carrageenan solution in drinking water prepared from carrageenan powder is used on the intestinal tract of pigs, this reduces the number of anti-inflammatory bacteria in the gut. Three known forms of carrageenan, kappa (κ), iota (ι), and lambda (λ) differ in degree of sulfation, viscosity, solubility, and gel-forming ability but have a similar backbone of β-1,2-linked D-galactose. All three isomers have shown comparable activity in inducing colitis in C57BL/6J mice model (Munyaka et al. 2016; Shang et al. 2017; Mi et al. 2020).

Indomethacin-Induced Colitis Indomethacin is a non-selective COX inhibitor that is used to produce enterocolitis in the rat model. It causes epithelial damage by causing a reduction in the synthesis of protective prostaglandins (PGE1 and PGE 2). Increased intestinal permeability, mucosal ulceration, and inflammation were observed in this model. *Bifidobacterium lactis* Bb12 (Bb12) have not shown any protective effect against ulceration induced in this rat model while *Lactobacillus rhamnosus* GG (LGG) exacerbated the intestinal injury. Naringin attenuated symptoms of colitis in rabbit model by reducing mucosal damage and expression of cytokines (TNF-α) (Menozzi et al. 2009; Kamil et al. 2007; Pawar et al. 2011; El Naggar et al. 2020).

Dextran Sulfate Sodium Induced Colitis DSS has been used to induce IBD in various studies by adding DSS in the drinking water of rats for 7 days. Induction of disease is confirmed by observing stool traits including the presence of bloody stool. Disease activity index (DAI) is calculated by formula: (weight loss score + stool trait score + blood in the stool)/3. Histopathology is used to measure the pathology injury score on the distal colon (Guo et al. 2020). Probiotics *L. acidophilus* CMUL067 and *L. Rhamnosus* IPL A2.21 have shown a protective effect against DSS induced colitis model (Zaylaa et al. 2018). *B. bifidum* ATCC 29521 has been widely investigated as it promotes epithelial barrier function, reduced bacterial translocation (Din et al. 2020). *Lactococcus lactis NZ9000 and Lactococcus lactis subsp. lactis* JCM5805 had also shown protective effect by alleviating symptoms of colitis, for example, loss in body weight and decreased length of colon (Komaki et al. 2020; Zeng et al. 2020). Cheese containing probiotics *Lactobacillus delbrueckii* subspecies. *lactis* CNRZ327, *Propionibacterium freudenreichii* CIRM-BIA 129 (equivalent to ITGP20 strain) and *Streptococcus thermophilus* LMD-9 have also been explored to treat symptoms of DSS induced colitis, it attenuated body weight loss and decreased DAI (Rabah et al. 2020). A dose of 1×10^8 cfuof *Streptococcus thermophilus* (NCIMB 41856) prepared in 3% sodium bicarbonate given by oral gavage reduced symptoms of DSS induced colitis. Also, colon length, mucosal

ulceration, loss of cryptal structure in the colon were restored. Inflammatory markers, e.g. IL-6, IL-17 were also reduced (Bailey et al. 2017). The probiotic complex comprising of *Lactobacillus spp., Streptococcus spp., Bifidobacterium spp.,* prebiotics, rosavin (extracted from *Rhodiolarosea L.*), and zinc caused decreased production of IL-1β, IL-6, and IL-17 (proinflammatory cytokines) and IL-10 (an anti-inflammatory cytokine) in DSS-induced colitis in mice (Park et al. 2018). A similar study was performed in specific pathogen-free (SPF) C57BL/6N mice, the bacterial strains *B. coagulans* RAM1202, *L. reuteri* RAM0101, *C. butyricum* RAM0216, and *B. longum* CICC6197 were given by oral gavage at a ratio of 1:1:1:1. The probiotic mixture alleviated symptoms associated with DSS-induced colitis (Wang et al. 2020). The probiotic strain of *Lactococcus lactis* subspecies. *lactis* not only increased the survival rate of mice but also improved histomorphic architecture of colon in DSS-induced colitis mice (Komaki et al. 2020).

Trinitrobenzene/Dinitrobenzene Sulfonic Acid Induced Colitis Intra-rectal administration of TNBS at a dose of 105 mg/kg can induce colitis in BALB/c mice. Wallace scoring method is used to assess the intensity of inflammation and the extent of lesions (thickening of the intestinal wall, intensity of ulceration, and colonic necrosis). Bacterial strains used for protection against this colitis, e.g. B. bifidum IPL A7.31, *L. rhamnosus* IPL A2.21 and *L. gasseri* IPL A6.33 (Zaylaa et al. 2018). Telmisartan also showed improved histopathologic changes in colon induced by TNBS (Arab et al. 2014).

DNBS-induced by rectal administration of colitis: 4% DNBS in 50% ethyl alcohol for two days in NC/Nga mice. DNBS group showed higher colon expression of the cytokines inducible nitric oxide synthase (iNOS) and cyclooxygenase(COX)-2 compared to the control group. *Enterococcus faecalis*, a gram-positive commensal bacterium has shown some protective effect. EF-2001 is isolated from feces of healthy human infant and it is one of the commercially available probiotics (Choi et al. 2019).

Citrobacter Rodentium Induced Colitis Model This is a bacterial incitant-induced model in which BALB/c mice are infected by oral gavaging them with resuspended *C. rodentium* (approximately 2×10^9 cfu) in PBS for continuous 14 days. The model induction can be confirmed by measuring levels of inflammatory markers, viz. TNF-α, IL-4, IFN-γ, and IL-12 in single-cell suspensions of mice colon. Probiotics combination of *L. acidophilus* ATCC 4356, *L. Plantarum* A *and L. Rhamnosus* ATCC 53103 which was referred to as Mixture-2 have been tested against colitis induced in this model. Before induction of colitis, Mixture-2 was administered to mice by oral gavage at a dose of 10^9 cfu daily for 21 days that resulted in reduced inflammatory cytokines, thus, provided protection against colitis induced by *C. rodentium* (Jiang et al. 2016).

4.9.3 Adoptive Transfer Models or Immune-Mediated Model

CD45RB High Transfer Model Purified $CD4^+$ $CD45RB^{high}$ T cells from spleen transferred adoptively into the immunodeficient recipient SCID mice by intraperitoneal injection caused the loss of goblet cells, an extensive inflammatory cell infiltrate and epithelial cell hyperplasia. *B. bifidum* which is the most abundantly present in the gut flora of breast-fed infants has shown a protective effect against inflammation by inhibiting disordered T cell activation leading to the production of IFN-γ from the basolateral chamber of colonic epithelium. Co-transfer of IL-10-transduced $CD4^+$ cells prevented the induction of colitis by $CD4^+$ $CD45RB^{high}$ T cells which provides an idea for using these interleukins for treating patients in clinics. It is well known that IL-10 which inhibits activation of T cells, monocytes, and dendritic cells regulates immune responses of the intestine by limiting and finally terminating inflammatory responses (van Montfrans et al. 2002; Kim et al. 2007; Kanai et al. 2006).

CD3εTg26 Transfer Model In this transgenic mice model, abnormal functioning of thymus leads to a loss of natural killer and T cell function, due to overexpression of human CD3ε This transgenic model showed increased expression levels of IFN-γ, TNF-α, and IL-12 when exposed to wild-type bone marrow depleted of T cells and finally leading to Th1-mediated wasting and colonic inflammation that could mimic ulcerative colitis. Aberrant thymic selected T cells could induce severe colitis by causing alterations in thymic microenvironments (Holländer et al. 1995).

Heat Shock Protein (hsp) 60-Specific CD8 T Cells Transfer Colitis Model An intestinal pathology model induction could be done by using antigen (hsp)-specific CD8 T cells. This model provided a link between infections and autoimmune diseases. The inflammation caused by these T cells was concentrated around only small intestine which was dependent on the hsp60 presentation on MHC class I and caused an increase in TNF-α expression. Thus, this model is antigen-specific and autoimmune-related inflammation which mainly affects the small intestine (Steinhoff et al. 1999).

4.9.4 Genetically Engineered Models

4.9.4.1 Gene Knockout Models

Interleukin-10-Deficient Mice IL-10 is a well-known suppressor of TH1 cells and macrophage effector functions. Mice deficient in IL-10 gene can develop IBD by pathogenic bacteria *Helicobacter hepaticus* as they are more susceptible to infections. Probiotic *Lactobacillus* spp., viz., *Lactobacillus paracasei* and *L. reuteri* strains by oral gavage reduced gastric inflammation confirmed by decreased inflammatory markers, viz., TNF-α and increased commensal strains in colon observed by doing RT-PCR (Peña et al. 2005).

Colitis in $G_{i\alpha}2$-Deficient Mice Heterotrimeric G proteins play an important role in signal transduction via adenylate cyclase pathway. Targeted knockout of $G_{i\alpha}2$, subunit in mice resulted in chronic colitis showing symptoms like UC in humans. In vitro studies with T-lymphocytes isolated from $G_{i\alpha}2-$deficient mice showed increased proinflammatory cytokines (IL-2, IFN-γ, and TNF-α) that could be the result of a delay in thymocyte maturation and resulting functional abnormalities (Rudolph et al. 1995).

Multiple Drug-Resistant (MDR1) Gene-Deficient Mice MDR1 is a well-known gene as a cause of resistance to chemotherapy in cancer treatment. This gene is expressed in the intestinal epithelium and some hematopoietic cells. MDR1α gene-deficient mice developed spontaneous bowel inflammation (Panwala et al. 1998).

STAT-3 Knockout Mice STAT-3 (signal transducer and activator of transcription 3) is responsible for signal transduction of many cytokines and growth factors. Knockout of STAT-3 gene in macrophages and neutrophils of mice developed enterocolitis. There were increased levels of proinflammatory cytokines (TNF-α, IFN-γ, IL-1, and IL-6) after the systemic exposure of lipopolysaccharides (O'Shea 1997; Takeda et al. 1999).

4.9.4.2 Transgenic Mouse Models

Colitis in HLA B27 Transgenic Rats HLA B27 transgenic rats show spontaneous development of IBD which involves entire gastrointestinal tract, mainly colon and is characterized by crypt hyperplasia and mucosal infiltration of mononuclear cells. This model is used to study the effect of resident intestinal flora on gut inflammation. Germ-free rats that do not develop colitis can be colonized with selective bacteria to check the effect of specific strain (e.g., Bacteroides spp.) on the onset of gut inflammation (Hammer et al. 1990; Rath et al. 1999).

IL-7 Transgenic Mice Interleukin IL-7 has been reported to be upregulated in the patients of UC. It is known to be involved in the proliferation and differentiation of mucosal lymphocytes and is present in intestinal epithelial cells. Mice transgenic for IL-7 develop acute colitis by both infiltering neutrophils and lymphocytes or with infiltration of CD4+ T cells, which suggests the role of the immune system in intestinal pathology (Watanabe et al. 1998).

4.9.5 Other Animal Models Used to Study Intestinal Inflammation

Drosophila Model for Gut–Microbe Interactions *Drosophila melanogaster*, the common fruit fly is known as a good model for developmental study and innate immunity. Recently, it has been introduced to study gut–microbiota interactions to overcome the limitation of cost, complexity, and ethical concerns related to using mammal models such as mice. It is well-known that fruit fly feeds on rotten fruits which contain microbes and survives well for more than 2 months, which clearly

indicates that they have a strong defence system against these microbes. The fly's response to wound infection is also fast and efficient and is both local and systemic. The possibility of genetic manipulation and conservation of this defence response make drosophila as an ideal model to study intestinal pathology. But there are limitations to all the models used to study human pathophysiology, so it must be chosen carefully. Drosophila can be well-suited for studying epithelial regeneration induced by enterocyte and innate immune response. The combinatorial effect of cellular immunity and regeneration can be well studied in the mouse model.

IBD in humans is linked with a reduction in healthy microflora in the gut wherein fruit fly's intestinal pathology is linked with a reduction in antimicrobial peptides (AMP) expression in midgut. Some of the pathogens, for example, *S. marcescens, V. cholerae*, *Enterococcus faecalis,* and *P. aeruginosa* are involved in the disruption of fly's intestinal homeostasis.

The limitations associated with using fruit fly are (1) Fibroblasts containing lamina propria and immune cells are absent, which makes it difficult to study non-epithelial intestinal inflammation and T-cells involvement in IBD and other intestinal pathologies. (2) Notch activation pathway is involved in the differentiation of intestinal stem cells (ISCs) which is not the case in mammals. The activity of this pathway can still be assessed by evaluating ISCs proliferation (Apidianakis and Rahme 2011; Ha et al. 2009; Buchon et al. 2014). In another study, the role of dual oxidase (DUOX) which is a member of the intestinal nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is studied in gut inflammation. Genetic evidence clearly shows that gut epithelia of drosophila can show two types of responses: Immune deficiency pathway which controls the production of AMP and DUOX pathway which controls ROS (reactive oxygen species) production which is known to be microbicidal. Decreased DUOX production in drosophila is related to various microbiota related infections in gut indicating ROS production in the gut plays an important role in controlling bacterial colonies in the gut. Knock-down of DUOX in drosophila caused dysregulation in commensal bacteria and an abnormal presence of bacteria of different shapes and fungi (Lee and Kim 2014; Ha et al. 2009).

Pig Model Intragastric administration of DSS to the weaned pigs caused mucosal inflammation and epithelial erosion. In this study nutrition supplements such as conjugated linoleic acid (CLA) and n-3 polyunsaturated fatty acids (PUFA) have shown a protective effect against symptoms associated with colitis (Bassaganya-Riera and Hontecillas 2006). Enteric bacterial pathogen (*Brachyspira hyodysenteriae*) can also induce colitis like condition by causing inflammation in colon mucosa (Hontecillas et al. 2002).

A gnotobiotic (Gn) pig model HAS been used to study the effect of a low and high dose of *Lactobacillus acidophilus* in potentiating immune response by rotavirus vaccine. Fecal samples of Gn pigs showed colonies of *L. acidophilus* confirming colonization of *lactobacillu*s in gut. The incidence of diarrhea caused by a rotavirus was also reduced in these pigs (Wen et al. 2012).

Naturally, *Chlamydia suis* causes diarrhea, body loss, dehydration, weakness, variable fever, and anorexia of several days in young piglets. This agent has been used to develop Crohn's disease in both the young piglet model and gnotobiotic pigs (Van Kruiningen 2016).

Early exposure of healthy microflora to young piglets prevented negative effects related to DSS-induced colitis. This protective effect is mainly by virtue of deactivation of toll-like receptor (TLR) signaling which finally leads to the reduced inflammatory response. The intervention of fecal microbiota from the Tibetan pigs to recipient animals (Yorkshire piglets) prevented them from having inflammatory responses indicating Tibetan pigs have the strong disease-resistance capability. This further gives an idea of the capability of using fecal microbe transplantation technology to regulate innate immune responses (Xiao et al. 2017).

Guinea-Pig Model Trinitrobenzene sulfonic acid (TNBS) and Freund's complete adjuvant (FCA) with alcohol have been used to develop a model of Guinea pig ileitis. Intraluminal instillation of TNBS in 50% ethanol is a classical model of intestinal inflammation that well mimics the location, the nature of the damage, and the inflammatory mediators observed in patients with Crohn's disease (Miller et al. 1995; Seago et al. 1970).

Nematode Model *Invertebrate* hosts like nematode *Caenorhabditis elegans* have been used to study immune response in intestinal epithelial cells. Although the transcription factor NF-κB, the TLR adaptor protein MYD88, and other TLRs are not present in *C. elegans*, its immune system involves evolutionarily conserved pathways: p38 mitogen-activated protein kinase (MAPK), FOXO transcription factors, and β-catenin (Kumar et al. 2019; Pukkila-Worley and Ausubel 2012).

Zebrafish as a Model for Investigating Animal–Microbe Interactions

Zebrafish (*Danio rerio*) has gained the attention of the immunologists and oncologists as it is a unique animal model which is a combination of conserved vertebrate physiology which has small, rapidly developing, and transparent body plan. These characteristics enable controlled, high-throughput experimental schemes in dissecting animal–microbe interactions across a range of spatial and temporal scales (Brugman 2016). Owing to the transparency of Zebrafish over the initial weeks of life, it serves as a potential tool to perform live in vivo imaging of bacterial exposure, epithelial responses to injury, and intestinal macrophages (Chuang et al. 2019). Zebrafish larvae have now used to study IBD and GI diseases. In comparison with humans, the zebrafish has similarity in GI system, having pancreas, gallbladder, intestinal tract, and liver, with comparable absorptive and secretory functions (Hanyang et al. 2017; Østensen et al. 2006). Intake of a diet containing high-fat changes the intestinal microbiota which causes low-grade inflammation (Arias-Jayo et al. 2018; Ballanti et al. 2014). Intestinal inflammation has been induced for larvae using different chemicals (such as DSS, TNBS, glafenine) as well as for adults using TNBS, oxazolone (Brugman and Nieuwenhuis 2017).

Zebrafish has also been used to screen compounds for anti-inflammatory activity in drug discovery programs. Also, the easy feasibility of tracking genetics in zebrafish makes it easy to study the susceptibility of genes in IBD. Unknown compounds for their ability to reduce neutrophilic inflammation were screened in DSS and TNBS induced enterocolitis model. The pharmacological modulation of dopamine receptors by cabergoline also showed a reduction in nitric oxide-induced inflammation in the TNBS-induced colitis model. Pharmacological modulation by cholecystokinin receptors by sincalide treatment decreased inflammation (Oehlers et al. 2017).

4.10 Clinical Status of Probiotic Interventions Studied Under Different Conditions

Healthy Adults In a clinical trial of 30 human healthy volunteers containing 1:1 sex ratio with the age between 23 and 43 years old treated with fermentation product with two probiotic strains, *Lactobacillus coryniformis* and *Lactobacillus gasseri* showed normal blood (blood biochemistry and the hemogram, total cholesterol, HDL-cholesterol and triacylglycerides plasma concentrations) and fecal parameters (lactic acid bacteria concentration, Random Amplification of Polymorphic DNA detection (RAPD) of the probiotic strains) throughout the study. Moreover, the probiotic combination increased hematocrit and red blood cell count along with increment in lactic acid bacteria in feces. Probiotic products caused an increase in frequency and volume of stools, thus, helps in improving bowel habits (Olivares et al. 2006).

Inflammatory Bowel Diseases IBD mainly constitutes pouchitis, UC, and Crohn's disease. Dysbiosis is defined as the disturbance in the bacteria flora between disease-promoting and protective strains and is considered as the major factor in the pathogenesis of IBD (Heilpern and Szilagyi 2008). Twenty six patients with CD, 31 patients with active UC, and 46 healthy volunteers were used to study microbial flora of colon mucosa using 16S rDNA based single-strand conformation polymorphism (SSCP) fingerprint, cloning experiments, using 16S rDNA based SSCP fingerprinting, oligonucleotide hybridization, and real-time polymerase chain reaction (PCR). This analysis showed the role of bacterial colonies in the induction of mucosal inflammation in the patients of IBD and supports the evidence that probiotics could be used to treat gut inflammatory diseases (Ott et al. 2004).

Crohn's Disease *Bifidobacterium longum* isolated from healthy human volunteer and prebiotic synergy 1 (containing oligofructose and inulin) showed efficacy to reduce disease activity index and histological score in a randomized, double-blind placebo-controlled trial on patients suffering from CD. The levels of proinflammatory cytokines such as TNF-α were decreased with the symbiont supplementation whereas there was no significant reduction in mucosal IL-18, INF-γ, and IL-1β as measured by RT-PCR (Steed et al. 2010). Intestinal permeability of

patients with CD in remission was decreased using *Saccharomyces boulardii* (probiotic yeast) as shown by reduced lactulose/mannitol excretion (Garcia Vilela et al. 2008). In another study by Bourreille, Arnaud, et al. demonstrated that *S. boulardii* could not stop the risk of relapses in CD patients and there was no improvement in Crohn's disease activity index (CDAI) in patients treated with *S. Boulardii* vs. placebo (Bourreille et al. 2013). Therefore, the efficacy of probiotics or symbiont against remission of CD cannot be concluded due to conflicting results. Future studies are required to confirm their effect on CD.

Ulcerative Colitis To maintain remission in UC, a single or combination of probiotics have been investigated. In a study by Venturi et al., probiotic formulation VSL# containing *bifidobacteria*, *lactobacilli,* and *streptococcus* strains was tested in patients who were allergic to 5-ASA treatment. The microbial flora was counted in fecal samples of patients and it showed healthy bacterial colonies as compared to the patients receiving placebo treatment. Thus, VSL#3 could be used to prevent the remission in UC patients (Venturi et al. 1999). Single probiotic *Escherichia coli* Nissle 1917 (EcN) treatment prevented remission in patients of UC and was comparable to gold standard mesalazine used to maintain remission in UC patients (Kruis et al. 2004). Still, there is a need to do different trials to study the effect of different individual probiotics and their combination to be used in IBD.

Pouchitis It is a common complication in 40–50% of patients of UC who undergo ileal pouch-anal anastomosis (IPAA) surgery. Its treatment mainly involves alterations in the anatomy of the bowel leading to change in the bacterial composition which gets exposed to the ileum. Fecal microbiota transplant (FMT) has shown efficacy against UC and *Clostridium difficile* infection. In the case of pouchitis, it showed a good safety profile and decreased bowel movement frequency and abdominal pain (Selvig et al. 2020). Gosselink et al. tested probiotic strain *Lactobacillus rhamnosus* GG to delay pouchitis in patients who recently underwent ileal pouch-anal anastomosis (IPAA) following UC. This study showed use of probiotics for the prevention of pouchitis onset and relapse (Gosselink et al. 2004). Gionchetti et al. showed the efficacy of VSL#3 versus placebo for the prophylaxis of chronic pouchitis using a randomized double-blind study on patients. VSL#3 is a probiotic preparation containing four strains of *Lactobacillus*, three strains of *Bifidobacterium*, and one *Streptococcus* (Gionchetti et al. 2003).

Functional Bowel Disorders (FBD) A double-blind clinical trial conducted on 68 patients suffering from FBD consisting of both males and females of age ranging from 18–65 years showed the efficiency of a probiotic combination of *Bifid bacterium lactis* and *Lactobacillus acidophilus* consumed daily prevented constipation in these patients (Ringel et al. 2011).

Gastroenteritis It is very common among children of age between 3 months to 12 years in the USA. A randomized double-blind trial conducted at a multicenter demonstrated that the probiotic product containing *Lactobacillus rhamnosus* and

L. Helveticus for 5 days did not prevent the development of gastroenteritis in young children (Freedman et al. 2018). Three-Combination Probiotics Therapy containing *Bacillus mesentericus, Enterococcus faecalis,* and *Clostridium butyricum* for seven days treated gastroenteritis of both *Salmonella* and rotavirus origin in pediatric patients (Huang et al. 2014).

Antibiotic-Associated Intestinal Disorders Diseases requiring antibiotic therapy, for example, respiratory infections, spinal cord injury (SCI), etc. may lead to antibiotic-associated diarrhea (AAD) which is defined as increased frequency (3 or more times) of water stools for 3 or more days (Bristol Stool Scale). A double-blind randomized placebo-controlled study on patients of age ranging from 16–75 years who were suffering from SCI showed less incidence of AAD when treated with multispecies antibiotics (Faber et al. 2020). Another similar study on children suffering from respiratory infections, probiotic (*Lactobacillus GG*) treatment before starting the course of antibiotics prevented the incidence of AAD (Arvola et al. 1999). A meta-analysis performed by Lynne V. McFarland also concluded the efficacy of different probiotic mixtures in the prevention of AAD and treating *Clostridium difficile* disease (McFarland 2006).

Colon Cancer It is the second to third leading cancer in Western industrialized countries. In some epidemiological studies, it was observed that the incidence of colon cancer decreased in a population consuming yogurt or fermented dairy product containing probiotics (*Lactobacillus* or *Bifidobacterium*) indicating probiotic could decrease the risk of colon cancer (Rafter 2004). A meta-analysis performed by Xiaojing, et al. showed the potential efficiency of probiotics in preventing infections after colorectal surgery in cancer patients. A randomized double-blind on 52 patients of colorectal cancer demonstrated the efficacy of consumption of probiotics in reducing infection post-surgery. Modification of intestinal bacteria resulted in a decrease in the proinflammatory markers like TNF-α and interleukins (IL-6, IL-10, IL-12) (Zaharuddin et al. 2019).

Necrotizing Enterocolitis in Infants Daily feeding of a probiotic combination containing *Bifidobacteria infantis, Streptococcus thermophilus,* and *Bifidobacteria bifidus to the neonates* of $\leq$1500 g birth weight showed effectiveness in reducing the incidence and severity of enterocolitis (Bin-Nun et al. 2005).

4.11 Conclusion

With an increase in the prevalence of intestinal inflammatory diseases, there is a need to do more investigations to deduce various causes and treatments for these diseases. Various factors can contribute to intestinal diseases. The initiation and progression of the disease are highly related to the interactions between the host and the gut microbiota. The entire chapter encompasses the importance of gut microbiota, prebiotics and probiotics and the relationship between gut microbiota and diseased

condition. The chapter particularly discussed the different factors and their possible mechanism in the development of inflammatory bowel disease. The plethora of animal models to study the mechanisms involved in gut inflammation are also discussed in detail. The most commonly used model systems are rodent models and particularly, genetically modified mice. The use of chemicals and bacterial incitants has fastened the development of intestinal inflammation; otherwise, natural inflammation generation is a time-consuming process. These incitants could cause tissue injury and related immune response in the animal model. Therefore, the use of animal models, different chemicals, or bacterial incitants has helped the investigators to understand the complex pathology of intestinal inflammation and the relation between the host and intestinal microbiota. Furthermore, it will then help to develop drugs and targeted therapy. Probiotics, prebiotics, and symbionts have shown their efficacy to prevent remission and relapse of IBD in preclinical models. However, clinical trials conducted by different research groups provide conflicting results that need further clarification. Future double-blind placebo-controlled studies showing efficacy and safety of probiotics are required before recommending its regular use for the treatment of IBD.

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Probiotics as Anti-Inflammatory Agents in Inflammatory Bowel Disease and Irritable Bowel Syndrome

5

Vasudha Sharma and Pritika Sharma

Abstract

Studies involving manipulation of intestinal microflora with probiotic species have suggested potential effectiveness in multiple gastrointestinal diseases including inflammatory bowel diseases. Resulting benefits of probiotics include competitively excluding pathogens, improvement of mucosal barrier function, and hence maintaining the gut homeostasis and modulating the immune system by inducing the release of cytokines. Few lactobacillus strains are also known to modulate the perception of pain by inducing effects similar to that of morphine in the intestinal epithelial cells. Probiotic products like VSL#3 (*Bifidobacterium longum*, *B. breve*, *B. infantis*, *Lactobacillus plantarum*, *L. acidophilus*, *L. paracasei*, *L. bulgaricus*, *Streptococcus thermophilus*), Culturelle (*L. rhamnosus* GG), Florastor (*S. boulardii*), Align (*B. infantis*), DanActive (*L. casei*), Mutaflor (*E. coli* Nissle 1917) are current examples of treatments. Probiotics are also increasingly being added in dairy as well as non-dairy products like drinks, yoghurts, etc. Most studies regarding the anti-inflammatory effects of probiotics are preliminary and seem promising but require further clinical trials. There is also scope for studies on the dosage, duration of therapies, ways of administration, and strain combinations.

Keywords

Anti-inflammatory probiotics · Inflammatory bowel disease · Irritable bowel syndrome · Ulcerative colitis · Gut microbiota

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5.1 Introduction

Inflammatory bowel diseases (IBD) cause different forms of inflammation in both the small intestine and colon. More than five million people have been reported to be affected worldwide, by IBD. IBD include chronic immune-mediated gastrointestinal disorders, mainly Crohn's disease (CD) and ulcerative colitis (UC). Although the exact causes of these diseases are unknown, most experts hypothesize that complex inflammatory response to environmental triggers results in IBD. Growing evidence suggests that, when the dynamic equilibrium of gut bacteria and mechanisms for defense in the host, at the intestinal mucosa (dysbiosis) gets disrupted, it may set off an inflammation reaction. Gastrointestinal tract beginning from the oral cavity and ending at anus is affected in Crohn's disease, whereas in case of ulcerative colitis, the large intestine and rectum are affected. Figure 5.1 represents the triggering factors for IBD.

When the difference between CD and UC with the help of absence of standard golden test becomes difficult, the third type of inflammation of the bowel is believed to have emerged which is known as inflammatory bowel disease unclassified or alternatively indeterminate colitis (IC). IBD is the most common between 15 and 40 years age group, although it can affect people of any age. Among patients

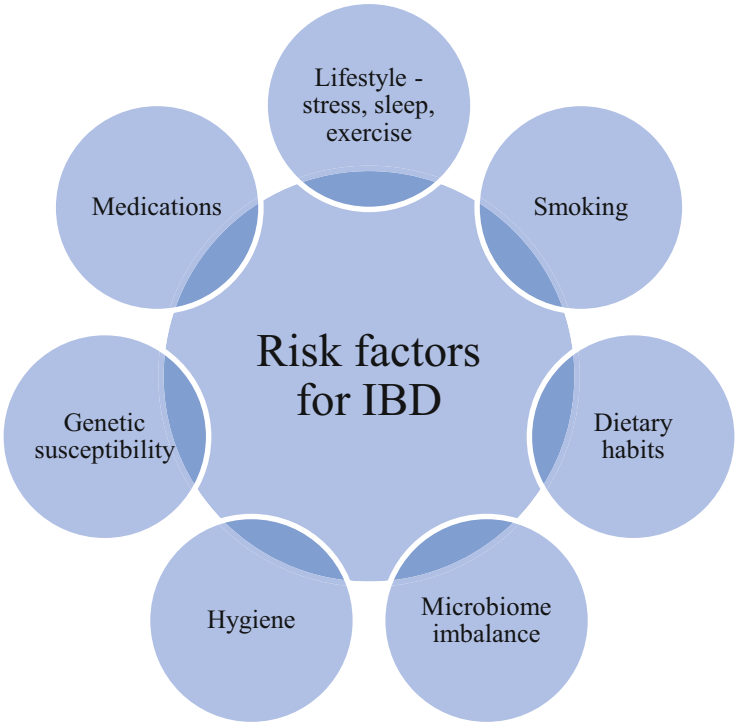

Fig. 5.1 Risk factors for IBD

Table 5.1 Difference between ulcerative colitis and Crohn's disease

Ulcerative colitis	Crohn's disease
First case reported in Europe in the year 1875	First case reported in the USA in the year 1932
Affects primarily colon	Affects various GI sites
Continuous inflammation of colon	Healthy and inflamed areas mixed
Affects innermost lining of colon	Can affect all layers of the bowel wall

suffering from IBD, about 7–20% are children and about 65–80% are people below 40 years of age. UC occurs at later years of life in people who smoke compared to patients who don't (Mahdi 2018). Table 5.1 shows the differences between ulcerative colitis and Crohn's disease.

Conventional treatments employed for IBD target inflammation and focus on suppressing the enhanced immune response with antitumor necrosis factor antibodies, steroids, and thiopurines. However, these treatments may result in serious and adverse health effects linked with chronic suppression of the immune response. Therefore, a better understanding of the pathophysiology of the disease has helped to propose alternative therapies by investigators, which focus on restoring the balance of the gut microbiota and eliminate bacterial antigens (Veerappan et al. 2012).

Irritable bowel syndrome, however, is a functional condition of the bowel in which individuals suffering experience altered bowel habits, abdominal pain with either diarrhea and/or constipation. It is diagnosed clinically as no biomarker has been found till date (Canavan et al. 2014).

5.2 Gut Microbiota

A dynamic and metabolically active ecosystem, the gut microbiota, serves a crucial functioning well-being and health. As revealed by researches on genotypic sequencing it has been demonstrated that the human gut can harbor between any of 1000 and 1150 varied species, with persons having at least 160. However, of these, 18 species were found in all participants of the study and 57 species were present in 90% of the participants, showing substantial dominance and stability between different individuals, of these microbes across humans (Zhu et al. 2010). Several factors such as age, disease, and food habits have an effect on the constitution of the gut microbiota. Alteration in the gut microbiota composition such as an increase in the levels of pathogenic bacteria and reduced levels of *Lactobacilli* and *Bifidobacteria* is associated with a range of gastrointestinal disorders (Guarner et al. 2012; Panghal et al. 2018). The human microbiota undergoes several changes depending upon the diet, environmental factors, use of medication, and intestinal transit time; however, it demonstrates a tendency to restore to the original composition, a phenomenon termed as resilience (Caporaso et al. 2012). A research study carried out using molecular biology techniques, with adults from Europe, Japan, and North America revealed that the composition of the gut microbiota is constituted predominantly by

3 "enterotypes," recognized by variation in the population of *Bacteroides* spp. (enterotype 1), *Prevotella* spp. (enterotype 2), and *Ruminococcus* spp. (enterotype 3) (Arumugam et al. 2011). Host immune response is largely affected by the gut microflora and therefore, manipulation of the composition of gut microbiota can help in amelioration of these gastrointestinal disorders (Celiberto et al. 2017).

5.3 Probiotics

Probiotics are microorganisms that provide beneficial and desired impacts on human health. The Food and Agriculture Organization of the WHO has defined a probiotic as "a live organism that, when ingested in adequate amounts, exerts a health benefit to the host." The microorganisms commonly used as probiotics are *Lactobacilli*, *Bifidobacteria,* and non-pathogenic yeasts such as *Saccharomyces boulardii*. Essential requirements for these microbes are that they should carry the ability to survive low pH due to stomach acid and bile and maintain the viable counts through the storage period and importantly should be safe for human consumption as depicted in Fig. 5.2 (Vasudha and Mishra 2013).

Fuller used the term "probiotic" for the first time and described it as "a live microbial feed supplement which beneficially affects the host animal by improving its microbial balance." Marteau et al. (2002) defined probiotics as "microbial cell preparations or components of microbial cells that have a beneficial effect on the health and wellbeing." Several mechanisms explain the beneficial action of probiotics for positively affecting the host. Probiotics prevent as well as ameliorate the condition in inflammation, by affecting the host immune response. Probiotic bacteria dwell at the binding sites of the gut mucosa and hence prevent the pathogens from adhering to it. *Lactobacilli* also secrete some proteinaceous compounds, such as bacteriocins, which work as local antibiotics in opposition to many pathogens and also thereby decreasing the production of pro-inflammatory cytokines. IgA is produced and is also stimulated by probiotics. *Lactobacilli* cause inhibition in the growth of bacterial pathogens by producing lactic and acetic acid. Probiotics also compete with the pathogens for nutrients and therefore, modify toxins that are produced by the pathogens found in the gut. It has been demonstrated that specific

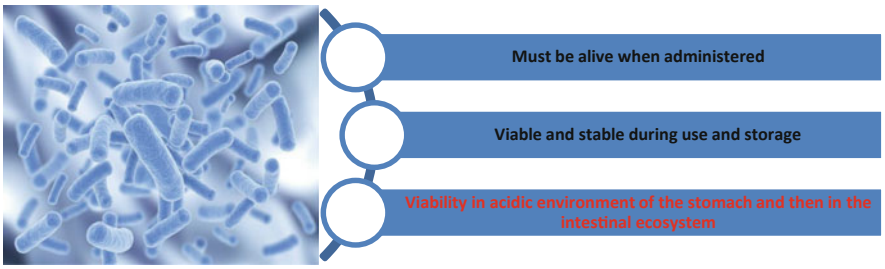

Fig. 5.2 Probiotics according to World Health Organization

DNA extracted from probiotics (VSL #3) could reduce the experimental colitis in animals (Chermesh and Eliakim 2006).

A precondition for probiotics to be used is its efficacy and safety. The characteristics for efficacy of probiotics are resistance to the process of digestion, by enteric enzymes, bile and gastric acids, and pancreatic enzymes, and the ability of preventing the pathogen binding, adherence, and multiplication of pathogens inside the gastrointestinal tract. Many types of bacteria qualify as probiotics such as lactobacillus family, but all bacteria cause different types of actions in different forms of diseases, some of the diseases can be better treated with combinations of these bacteria and their dosing is also an issue. Hence, probiotic treatment can be considered relatively safe but not as completely risk free (Chermesh and Eliakim 2006).

5.4 Pathophysiology of the Disease

5.4.1 Inflammatory Bowel Disease

Normal mucosal integrity is disrupted due to inflammation anywhere in the gastrointestinal tract. Therefore, IBD is very painful and can be potentially fatal in several cases. The symptoms include pain in the abdomen with cramps and swelling in stomach, bloody diarrhea, fatigue, fever, weight loss, vomiting, and anemia. Other symptoms which are sometimes seen include pain in the joints, red and painful eyes, skin nodules, and jaundice. These symptoms relapse characteristically (Mahdi 2018). Recent studies implicate that an imbalance of the gastrointestinal microbiome can be a potential trigger in case of IBD. Use of probiotics as a supplement alongside standard anti-inflammatory therapy is gaining interest and popularity. Potential mechanisms of action using animal models suggested that probiotics can reduce inflammation in the colon.

Distinct differences exist in the histopathology of the two main types of IBD: Crohn's disease and ulcerative colitis. The whole intestinal segment is affected, interspersed with some healthy areas in the case of CD and this is a case of chronic inflammation. The terminal ileum and colon are affected in most cases and it begins with short duration of intense occurrence of diarrhea, fever, weight loss, and repeated abdominal pain. Whereas, in ulcerative colitis, the inflammatory reaction demonstrates distinct characteristics such as the occurrence of abscesses in the crypts and permeation of eosinophils, neutrophils, and plasma cells that attack the lining of the colon and rectum repetitively. Common symptoms in the case of individuals suffering from UC are diarrhea, bleeding from rectum, mucous discharge, and abdominal pain. Figure 5.3 shows images of affected areas of the human body in the case of Crohn's disease and ulcerative colitis.

People of all age groups are affected by IBD; however, a higher chance of occurrence is observed in people between 15 and 30 years of age and elderly population. Several systematic scientific investigations in the past few years have revealed an increased incidence of IBD among nations where the socio-economic status is on the rise (Celiberto et al. 2017).

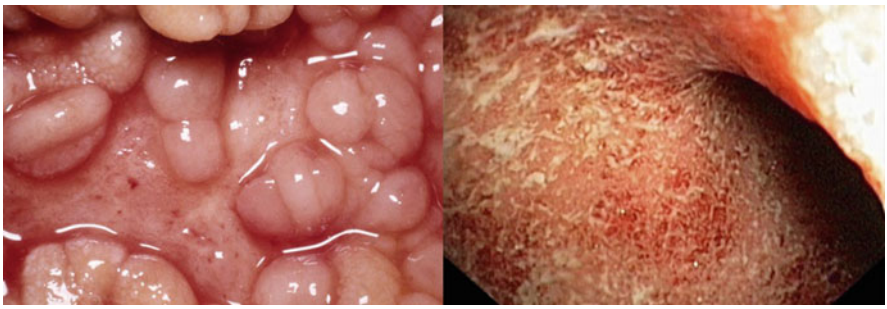

Fig. 5.3 Areas affected by (1) Crohn's disease and (2) ulcerative colitis [Source: https://www.webmd.com/ibd-crohns-disease/ss/slideshow-inflammatory-bowel-overview]

Probiotics play a role in immunomodulation and anti-inflammatory response and are used in the treatment of chronic diseases. A study by Mack (2011) conducted a systematic review on the outcomes of consumption of probiotics on chronic intestinal diseases occurring in animals and humans. Probiotic strains decreased the expression of pro-inflammatory cytokines through a mechanism which is chiefly mediated by toll-like receptors. Probiotics' administration bettered the condition of the disease and the histological changes in maximum animal studies, but few results also suggested that care needs to be exercised when probiotics are administered, in cases of relapse of IBD. Probiotic supplementation seems to have potential and is safe for individuals suffering with UC and CD. Clinical symptoms were improved by the use of *Bifidobacterium longum* 536 in patients suffering from mild and moderate UC (Mack 2011).

Although the data present is not enough to recommend probiotics for remission in UC and CD, there is enough evidence that supports that probiotics can be employed for diminution of severity in pouchitis. The regulatory standards for probiotics are insufficient but probiotics have minimal side effects according to reports and are hence relatively safe. A higher number of in-depth studies are required for supporting the efficacy and safety of these, before they can be used as treatment in IBD. Figure 5.4 represents the mechanism of symptoms in IBD and IBS (Spiller and Major 2016).

5.4.2 Irritable Bowel Syndrome

Bloating, abdominal pain, and a change in the stool frequency are symptoms observed in the case of irritable bowel syndrome. It is a challenging illness which results in a deteriorated quality of life. The prevalence of IBS is between 10 and 20% in developed nations, and increased absence and excessive utilization of healthcare services result in insignificant economic consequences. Many factors such as genetics, gastrointestinal microbiota, behavioral pathways, and abnormal pain processing serve a crucial role in pathogenesis of the disease. A large case controlled research

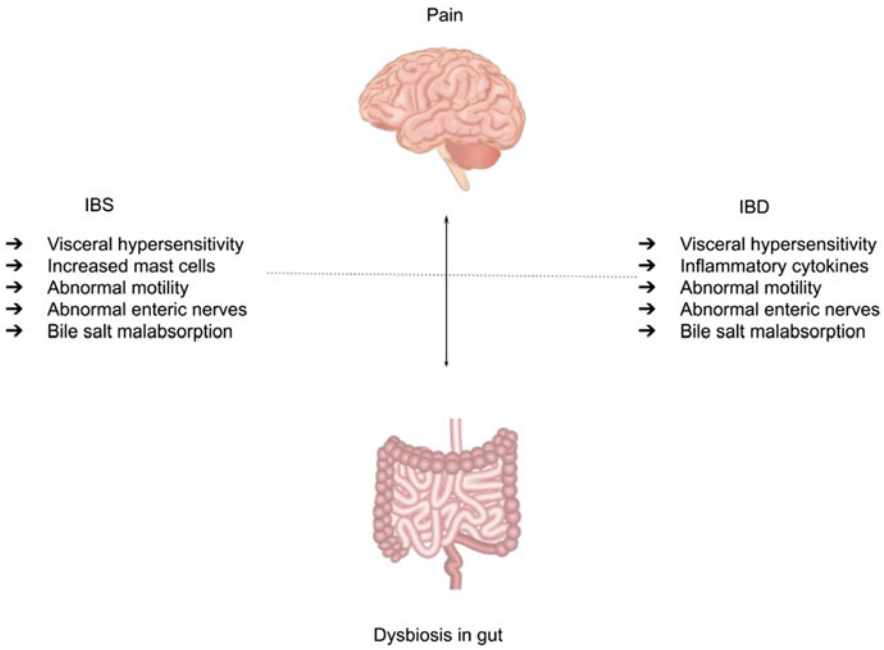

Fig. 5.4 Mechanism of symptoms in IBS and IBD

revealed that infectious gastroenteritis resulted in almost four times increase in the chances of developing irritable bowel syndrome within the following 2 years (Whelan and Quigley 2013). Many researches have informed a luminal dysbiosis in IBS, with many patients showing decreased counts of *Lactobacilli* and *Bifidobacterium*; these microbes are generally used in probiotic products.

5.5 Probiotics: Mechanisms of Action

Probiotics include a few symbiotic bacterial flora, having beneficial effects on host health and also prevent disease or help in its treatment. Probiotics have shown beneficial effects on several human diseases such as inflammatory bowel diseases (IBD), during clinical trials. However, clearly understanding and establishing the mechanism of action is an important matter related to the usage of probiotics clinically.

5.5.1 Inflammatory Bowel Disease

Essentially, research studies highlight three different molecular and cellular mechanisms for regulation of probiotic therapy in IBD, as discussed below:

(a) Bactericidal substances are produced by probiotics, which obstructs the effects of pathogenic bacteria and causes competitive inhibition in case of the pathogens and toxins in their ability to adhere to the intestinal epithelium;
(b) Immune responses can be regulated by probiotics by improving the intrinsic immunity and modifying inflammation induced by pathogens via signaling pathways that are regulated by receptors; and.
(c) Probiotics improve intestinal epithelial homeostasis by enhancing cell survival in intestinal epithelium, stimulating defense responses and improving barrier function. Probiotics regulate host cell by signaling pathways, including mitogen-activated protein kinases, Akt and nuclear factor-κB to arbitrate the role of the intestinal epithelium.

Developing an understanding of the mechanism of probiotic action will help in forming the rationale, in support of further studies, with new hypothesis to explain the clinical effectiveness in preventive and alternative treatments in case of IBD (Ng et al. 2008).

5.5.2 Irritable Bowel Syndrome

Many studies point towards the positive health effects of probiotics in IBS. First and foremost, numerous probiotic microorganisms exhibit antiviral and antibacterial effects and could therefore check or alter the path of IBS, postinfection. Moreover, probiotics have been successfully demonstrated to exhibit anti-inflammatory effects at surface of mucosal membrane; by decreasing its inflammation, probiotics can reduce immune-mediated activation of enteric motor and sensory neurons and modify the patterns of nerve activity between the gut and the central nervous system. Third, possible mechanism of action of probiotics could be by modifying the composition of the gastrointestinal microflora (Oelschlaeger 2010). The position of gut flora in IBS is still a point of disagreement and lacks consensus; however, probiotic-related variation in the intestinal microflora could straight away (via the escalation of commensals, i.e., *Lactobacilli* or *Bifidobacteria*, or the exclusion of disease causing microbes) or in turn (by way of reducing either pathogen-related inflammation or bacterial fermentation) affect the functioning of the gut. Lastly, probiotics could modify the composition and/or volume of stool and gas or cause an increased intestinal mucus secretion; effects that could influence the handling of its contents in the intestine and thus transform symptoms such as diarrhea and constipation.

5.6 Biological Basis for Positive Action of Probiotics

Biological effects of probiotics that may help in amelioration of condition in inflammatory bowel disease and irritable bowel syndrome can be categorized as follows:

5.6.1 Pathogen Resistance

Probiotics are believed to maintain host–microbial homeostasis and therefore reduce attack by pathogens and their increased presence in the gut. If endogenic microorganisms inhabit all functional niches, the pathogenic invasion can be reduced. Probiotics can prevent opportunistic infection either by occupying functional niches which are exposed by internally originating community or they might modulate the environment by secreting short chain fatty acids (SCFAs), bacteriocins, reactive oxygen species, and lactic acid, thereby inhibiting the growth of pathogens.

5.6.2 Nutritional Process

Some species in the gut microbes contribute to availability of vitamin and produce SCFAs. Gut microorganisms can produce vitamin K and B_{12}, biotin, pyridoxine, folate, thiamine, and nicotinic acid. Also, butyrate is a major source of energy and also maintains the enteric mucosa.

5.6.3 Immune Process

Probiotics have been proved to have a number of positive effects on the immune system. Some probiotics are known to be immunostimulatory, they induce IL-12 and NK or natural killer. Some species are anti-inflammatory and induce IL-10 and regulatory T cell pathways. Specific probiotic strains have specific effects on the immune system, some act as immunostimulatory and some as anti-inflammatory agents.

5.6.4 Rectifying Contaminants

Some probiotics such as *Pediococcus pentosaceus* are known to lower the risk from intake of compounds that are hazardous. It disintegrates fumonisins that are a group of mycotoxins which are produced from fungi, *Fusarium* spp.

5.6.5 Drug Metabolism

Gut microbiota can play a pivotal part in metabolism of drugs that can consequently, positively impact the therapy for various disease conditions. Competitive inhibition of hepatic sulfotransferases can decrease the liver's capacity to metabolize paracetamol; however, gut microbiota may play a crucial role in its metabolism (Li et al. 2016). Moreover, there is a lot of evidence to prove that environmental chemical pollutants as well as dietary ones can interfere with functions of gut bacteria such as

transcription and metabolism, which can lead to an adverse effect on the host health, by inducing an immunostimulatory reaction inside the gastrointestinal system.

5.6.6 Bile Acid Metabolism

Deconjugation of primary bile acids and their conversion to secondary bile acids by some species of microbes in the gut like *Bacteroides intestinalis* are known. Secondary bile acids then hinder the germination of *Clostridium difficile* spores and hence restrict the increase of *C. difficile* (Day et al. 2019).

5.7 Microbiology Based Theories

Probiotics are considered to be safe as food additives, nutritional supplements, or pharmaceutical formulations. Studies suggest that dead microorganisms and their biologically active compounds play protective functions as well and therefore, the definition of "probiotic" should be revised or there should be implementation of classifications. Specific microbial strains have different mechanisms of action. Growth factors are produced by probiotic strains which help in strengthening the gut epithelium and antimicrobial substances such as SCFAs, hydroperoxides, bacteriocins, lactic and bile acids that help in killing harmful microorganisms. Consequently, cellular components get released inside the gut; this enhances the production of pro-inflammatory cytokines and activates immune responses and synthesis of immunoglobulin. It also improves lymphocytes and macrophage activity. Although immune tolerance is a plausible consequence of these improvements, there is little or no agreement in this regard. Benefits of probiotics other than immune improvements are improvement in the process of digestion and absorption, pH alterations, amalgamation of pathogens, and isolation of metabolic toxins. Animal models as well as in vitro assays suggest that decrease in apoptosis can also be achieved by probiotics along with increase in mucus synthesis, repair of tissues, and creation of tight linkages in epithelial cells in the gut, hence causing reduction in the permeability of intestine and enhancement of the barrier functions and its protection. *Lactobacillus* species (e.g., *L. rhamnosus*, *L. casei*, *L. plantarum*, *L. paracasei*, *L. acidophilus*, *L. johnsonii*, *L. reuteri*, *L. gasseri*) and Bifidobacterium (e.g., *B. bifidum, B. longum, B. infantis, B. animalis, B. lactis, B. breve, and B. adolescentis*) are the most commonly used probiotic strains in formulations, but an approach with combining the species is also being applied increasingly. Other strains that are used are Streptococcus spp., *Enterococcus* spp., *Lactococcus* spp., *Clostridium* spp., and non-pathogenic *Escherichia coli*. New genera of bacteria and species have also shown good insightful information in preclinical trials. New-generation probiotics is the name given to these bacteria. They are more complex than common probiotics in an effort to imitate fecal microbiota transplant treatments. These probiotics of the new generation include Clostridium clusters IV, XIVa, and XVIII, *Akkermansia muciniphila*, *Bacteroides uniformis*, *Eubacterium*

hallii, B. fragilis, and *F. prausnitzii*. Current challenges in the way of employing these new-generation microbes as probiotics are technological aspects. Clostridium clusters XIVa and IV boost the Treg differentiation process, which is significant in case of immune tolerance. Indeed, in the gut of IBD patients, these bacteria are decreased and Tregs are increased, the expansion is still insufficient in restraining the development of inflammation. Gastrointestinal microbiota does not only include bacteria, some studies suggest use of yeasts in their formulations as probiotics along with bacteria and even as single-drug formulation. *Saccharomyces boulardii* has many anti-inflammatory properties and is the most commonly used strain in this context (Basso et al. 2018).

5.8 Probiotics for Treatment of Gastrointestinal Diseases

The global market is continuously expanding in case of probiotic supplements. Although the public perceives probiotics as beneficial, the studies to conclude that probiotic strains can improve characteristics of disease are few. There is a lack of research trials on a larger scale and the understanding of the reciprocal action with the human system after supplementing with probiotics. For probiotics to render to routine healthcare more in-depth research of various strains individually and their response to application of advanced measurement techniques is required. This will provide useful data or use of probiotics in routine healthcare practice.

Health claims need to be specific in case of probiotics and European Food Safety Authority (EFSA) considers claims like "strengthens the immune system" to be too vague. A lot of research has gone into making a base of evidence that probiotics can have an impact on different biomarkers. For a clearly displayed cause and effect relationship at the minimum randomized, placebo-controlled clinical trials are required by EFSA. Research and development lacking in investment and fundamental knowledge and having a poor design of study leads to "pilotitis"—where small projects create insufficient evidence for regulatory approval. An example is the systematic study of randomized controlled trials which investigated effects of probiotics, in remission of IBS. 35 RCTs were reviewed, out of those 3 had minimum 100 participants, it was deduced that about 75% were just preliminary studies (Dignass et al. 2012).

Appropriately driven phase III clinical trials for probiotics are needed to establish and improve confidence in efficacy of product for clinicians and therapists (Basso et al. 2018).

5.8.1 Probiotics in IBS

Irritable bowel syndrome (IBS) is highly prevalent and affects about 3 to 15% of the general population. Its characteristics include unexplained pain in the abdomen, bloating, and discomfort along with changed bowel habits. Multiple causes have been discovered in pathophysiology of inflammatory bowel diseases. Along with

abnormal gastrointestinal motor function, it has been linked with visceral hypersensitivity, inflammation, autonomic dysfunction, and even psychosocial factors. According to a study, IBS developed in a subgroup of patients after an acute bacterial infection in the bowel. Inflammatory cells like the mast cells and lymphocytes in the mucosa of colon were found to have increased in IBS patients which suggested that these patients had an ongoing state of inflammation (Bennet et al. 2015). Probiotics can be beneficial in postinfectious IBS since they have shown to benefit in acute cases of inflammatory bowel disease as well as infectious diarrhea. Another reason in favor of using probiotics is that they can influence the process of fermentation and avoid gas production by changing the flora in the colon. Although there is less data to support the benefits of probiotic treatment in controlled clinical trials, still there is enough to support that they can normalize the levels of inflammatory cytokines and other symptoms of IBS. The comparison between various studies is complicated since the sample sizes are different and so are the probiotic bacteria used. Though the preliminary results look promising, the clinical efficiency and the precise mechanism of action in inflammatory bowel syndrome are to be studied with better designed experiments and controlled trials (Andresen and Baumgart 2006).

5.8.2 Probiotics in IBD

As mentioned earlier, ulcerative colitis and Crohn's disease are the two main types of inflammatory bowel diseases (IBD).

These are diseases of the gastrointestinal tract which are characterized by chronic inflammation. An imbalance between the intestinal mucosa and the commensal gut flora related to the immune system is the prime reason studied in the pathological process resulting in the development of these diseases. Theory suggests that probiotics can be utilized to treat IBD as they can interfere with the abnormal homeostasis that is a characteristic of IBD. They can hence reestablish the immune-bacterial interaction in the mucosa of the intestine. The mechanism of action includes reconstituting the composition of flora that is altered in case of IBD. They also help in enhancing the epithelial barrier's integrity. They promote the tolerance action by the accessory cells or the antigen-presenting cells along with strengthening the defense mechanism of the inborn immunity and suppression of adaptable immune responses that are capable of inflammation. There is not enough positive clinical evidence that supports the experimental evidence regarding the benefits of probiotics in IBD. Diversity of microorganisms that have been used for various trials and the varied dosages due to lack of standardization could be the reason for the discrepancies. Also, the scheme of administering the probiotics was different. The varied clinical phenotypes and the heterogeneity in the clinical trials are the important critical issues for the use of probiotic therapies optimally in case of patients suffering with IBD (Pagnini et al. 2013).

The number of randomized trials for consequence of probiotics is limited, in case of remission in ulcerative colitis and there are many differences in their methodology. The studies that exist until now have been about comparison of probiotics with

anti-inflammatory drugs. These studies suggest that the efficacy and safety of the two are comparable. An alternative is using the probiotics that cure inflammation by interacting with the host epithelium. The microfloral composition as well as metabolic activities can be modified using probiotics as they can prevent the growth of potential pathogens. Pathogenic bacteria are linked with inflammation. Rigorous research is needed in the complex field of probiotics. If bacteria contribute to the pathogenesis of ulcerative colitis and resistance to antibiotics, probiotics can offer a substitute mechanism to manipulate the microflora in continually occurring diseases. Many studies suggest that some selected probiotic preparations have a positive influence on gastrointestinal diseases including UC. The most widely used probiotics in humans are *Bifidobacteria* and *Lactobacilli*. However, information is based on relatively small studies, which are insufficient to establish if they are definitely useful, and the pros and cons implicated are still feebly comprehended.

There have been reports suggesting significant improvement with different strains in bacterial species and yeasts. As mentioned by Zigra et al. (2007) "studies regarding *Bifidobacteria* treatment in a control group were found to have a significantly better clinical effect, in comparison to those with *E. coli. Bifidobacteria vs* control group: odds ratio7.32 (1.37–39.13), *E. coli vs* control group: odds ratio 0.66 (0.43–1.02). The form of UC seemingly does not impact the outcomes: mild-to-moderate UC: odds ratio 3.39 (0.97–11.87), active UC: odds ratio 3.79 (0.37–39.01), nonactive UC: odds ratio 1.26 (0.64–2.46)."

5.9 Comparison of Probiotics with Anti-Inflammatory Drugs and Placebo

Among studies that compare the impact of probiotics with that of the placebo Zigra et al. stated that the "tests that collate the impact of probiotics with that of the placebo (*Bifidobacteria vs* placebo, symbiotic *vs* placebo) gave better results than studies that compared the effect of probiotics with the effect of anti-inflammatory drugs. Among five randomized, controlled studies comparing probiotics with anti-inflammatory drugs, one of the trials showed a trend for increased efficacy. The other four studies did not find any significant difference between probiotics and anti-inflammatory agents. The pooled relative risk was 0.95 (95% CI 0.58–1.55, $p = 0.84$), showing no significant difference between probiotics and treatment with anti-inflammatory drugs. A nonsignificant heterogeneity was found ($Q = 9.63$) as the normal heterogeneity for 5 df according to the $\times 2$ distribution was 9236. Among four randomized, controlled studies with probiotics with placebo, two trials reported significantly higher remission in UC for patients receiving probiotics. The other two trials showed a trend for augmented efficacy of probiotic in comparison with placebo. The pooled relative risk was 7.32 (95% CI 1.37–39.13, $p = 0.020$), showing a significant difference between probiotic and placebo."

Table 5.2 collates several clinical trials that demonstrate the effectiveness of probiotics in IBD and IBS.

Table 5.2 Clinical trials suggesting the effectiveness of probiotics in IBD and IBS

Author	Title	Study	Results
Kajander et al. (2008)	"Clinical trial: Multispecies probiotic supplementation alleviates the symptoms of irritable bowel syndrome and stabilizes intestinal microbiota"	"The effects of multispecies probiotic supplementation (*Lactobacillus rhamnosus* GG, *L. rhamnosus* Lc705, *Propionibacterium freudenreichii* ssp. *shermanii* JS and *Bifidobacterium animalis* ssp. *lactis* Bb12) were investigated on abdominal symptoms, quality of life, intestinal microbiota and inflammatory markers in irritable bowel syndrome"	"This multispecies probiotic seemed to be an effective and safe option to alleviate symptoms of irritable bowel syndrome and to stabilize the intestinal microbiota"
Enck et al. (2009)	"Randomized controlled treatment trial of irritable bowel syndrome with a Probiotic *E. coli* preparation (DSM17252) compared to placebo"	"Two hundred and ninety-eight patients with lower abdominal symptoms diagnosed as IBS were treated for 8 weeks by the compound Symbioflor®-2 (Symbiopharm GmbH, Herborn, Germany), an Escherichia coli product ($N = 148$), or placebo ($n = 150$) in a double-blinded, randomized fashion"	"Treatment of IBS with the probiotic Symbioflor-2 was effective and superior to placebo in reducing typical symptoms of IBS patients seen by general practitioners and by gastroenterologists"
Zocco et al. (2006)	"Efficacy of *Lactobacillus* GG in maintaining remission of ulcerative colitis"	"The efficacy of *Lactobacillus* GG alone or in combination with mesalazine vs. mesalazine was evaluated as maintenance treatment in ulcerative colitis"	"*Lactobacillus* GG seemed to be effective and safe for maintaining remission in patients with ulcerative colitis, and it could represent a good therapeutic option for preventing relapse in this group of patients"
Kruis et al. (2004)	"Maintaining remission of ulcerative colitis with the probiotic *Escherichia coli* Nissle 1917 is as effective as with standard mesalazine"	"Comparison of the efficacy in maintaining remission of the probiotic preparation *Escherichia coli* Nissle 1917 and established therapy with mesalazine in patients with ulcerative colitis"	"The probiotic drug *E coli* Nissle 1917 showed efficacy and safety in maintaining remission equivalent to the gold standard mesalazine in patients with ulcerative colitis"
Rembacken et al. (1999)	"Non-pathogenic *Escherichia* coli versus mesalazine for the treatment of ulcerative	"Aim was to find out whether the administration of a non-pathogenic strain of *E coli* (Nssle 1917) was	"Results suggest that treatment with a non-pathogenic *E coli* has an equivalent effect

(continued)

Table 5.2 (continued)

Author	Title	Study	Results
	colitis: a randomized trial"	as effective as mesalazine in preventing relapse of ulcerative colitis. It was also examined whether the addition of *E coli* to standard medical therapy increased the chance of remission of active ulcerative colitis"	to mesalazine in maintaining remission of ulcerative colitis"
Guslandi et al. (2000)	"*Saccharomyces boulardii* in maintenance treatment of Crohn's disease"	"Patients with Crohn's disease in clinical remission were randomly treated for six months with either mesalamine 1 g three times a day or mesalamine 1 g two times a day plus a preparation of *Saccharomyces boulardii* 1 g daily. Clinical relapses as assessed by CDAI values were observed in 37.5% of patients receiving mesalamine alone and in 6.25% of patients in the group treated with mesalamine plus the probiotic agent"	"Results suggested that *Saccharomyces boulardii* may represent a useful tool in the maintenance treatment of Crohn's disease"
Nobaek et al. (2000)	"Alteration of intestinal microflora is associated with reduction in abdominal bloating and pain in patients with irritable bowel syndrome"	"Patients were randomized into two groups, one receiving 400 mL per day of a rose-hip drink containing 5×10^7 cfu/ml of *Lactobacillus plantarum* (DSM 9843) and 0.009 g/mL oat flour and the other group receiving a plain rose-hip drink, comparable in color, texture, and taste. The administration lasted for 4 wk"	"The results of the study indicated that the administration of *Lb. plantarum* with known probiotic properties decreased pain and flatulence in patients with IBS"

5.10 Conclusion

Considerable substantiation data demonstrating that probiotics contribute to anti-inflammatory effects exists through established researches; however, mechanistic studies clearly bringing out the path by which they act are not clearly understood. It

is important not to generalize the health effects from specific strain studies to species effects, whether positive or negative. Most commonly, probiotics are to be used as supplements particularly for patients who do not respond positively to regular treatment regime. Major advantage of these probiotics is that they are tolerated well by the body with insignificant adverse health effects described in the aforementioned studies.

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Antibiotic-Associated Diarrhea and Update on Probiotics Recommendations

6

David Elisha Henry and V. Venkateswara Rao

Abstract

Antibiotic-associated diarrhea (AAD) is an adverse impact of antibiotic therapy which alters the metabolic function of the host's gut microbiota causing diarrhea (osmotic or infectious type), and significant infection by *Clostridium difficile*. *C. difficile* is an opportunistic pathogen which thrives in the gut when the colonization resistance conferred by gut microbiota is compromised, leading to pathogenesis ranging from mild diarrhea to serious conditions like pseudomembranous colitis, which could be fatal. The impact of antibiotic therapy on the composition of gut microbiota has been observed to extend beyond the clinically targeted bacterial species since removing populations of certain species of gut bacteria opens up niches for other microorganisms to colonize, subsequently resulting in gut dysbiosis. Multiple meta-analyses have elucidated the cumulative beneficial impact of orally administered probiotics for the effective prophylaxis of CDI. Probiotics also benefit the host by immunomodulation which is critical in management of inflammation in the gut. It has been hypothesized that AAD is caused by dysbiosis which probiotics beneficially modulate and assist in restoring the homeostasis of the unbalanced indigenous gut microbiota. Probiotics have been proven to reduce the risk of AAD by 51% without the risk of any adverse effects. The present chapter provides a comprehensive outlook on the current trends in the management of AAD by the intervention of various

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probiotic microorganisms, highlighting their merits and demerits to facilitate effective management of the dysbiosis of the gut and its allied metabolic and immunological ramifications that are critical factors contributing to the onset and development of AAD.

Keywords

Gut microbiota · AAD · Dysbiosis · *Clostridium difficile* · Probiotics · Immunomodulation · Metabolites

6.1 Overview

Antibiotic-associated diarrhea (AAD) is the most important variant of nosocomial diarrhea which is characterized by unexplained diarrhea observed in patients undergoing antibiotic therapy, subsequent to the exclusion of other possible aetiologies (Barlett 2002). Globally, the frequency of AAD varies widely ranging from 4.3 to 80% [median value: 22%], with the mean age of patients with AAD being in the range of 18–48 months. It has been observed that in children, the primary risk factors for AAD comprise the age of the child and also the specific type of antibiotic used (McFarland et al. 2016). The onset of AAD might take place within a range of a few hours or up to 8 weeks subsequent to the administration of antibiotics (Cote and Buchman 2006), and it contributes significantly to the aggravated suffering as well as contributing to additional costs and duration of hospitalization (McFarland 1998). The severity of AAD ranges from symptoms of mild diarrhea to progressing towards serious conditions such as fulminant pseudomembranous colitis (McFarland 2006). Children develop the symptoms much more rapidly compared with the adults. Remarkably, the recovery period in children is faster, and they also display lesser complications, and the overall duration of the disease is less, in comparison with adult patients. The specific mechanism of AAD is yet to be completely elucidated. Research reports have revealed that the putative mechanism of action for the onset of AAD is the direct impact of the administration of antibiotics on the gastrointestinal mucosa, which ultimately alters the composition of the gut microbiota and the proliferation of pathogenic microorganisms. Gastrointestinal disturbances are well documented as an adverse effect of administration of broad-spectrum antibiotics [e.g. Vancomycin, Amikacin, Gentamicin, and third generation β-lactams]. *Clostridium difficile* is the most prominent causative agent of AAD (Fox et al. 2015). However, other pathogenic microbes such as *Staphylococcus spp.*, *Candida* spp., members of Enterobacteriaceae (e.g. *Klebsiella*), may also contribute to AAD.

The incidence of CDAD (*C. difficile* associated diarrhea) cases has recorded exponential growth during the past decade, with a larger proportion of cases being reported as “community-acquired” type. Critical illness among hospitalized patients has been strongly associated with gut microbial dysbiosis, potentially aggravating the susceptibility to developing infection, ultimately leading to organ failure. The

risk of health complications linked to gut dysbiosis due to antibiotic therapy (viz. diarrhea) highlights the importance of rational administration of antibiotics (Kołodziej and Szajewska 2017).

The classic pathogenesis of CDAD is attributed to administration of antibiotics and the subsequent dysbiosis of the microbiota of the GI tract, ultimately causing colonization of the gut mucosa by *C. difficile*. Notably, the administration of anti-acidity therapy [viz. proton pump inhibitors (PPI) and H_2-receptor antagonists] has been linked to aggravated risk towards CDAD. One possible hypothesis is that the elevated pH in the gastric contents could enhance the survival of *C. difficile*. In addition, the administration of PPI, irrespective of the duration of treatment, has been shown to alter the gene expression in human colon cell lines, leading to decreased integrity of the colonocytes (Biswal 2014). Therefore, a possible association has been proposed regarding PPIs and nonsteroidal anti-inflammatory drugs (NSAIDs), viz. diclofenac, and community-acquired CDAD in patients without a recent history of hospitalization or exposure to antimicrobial compounds has been hypothesized (Permpalung et al. 2016).

Imperatively, there is an ambiguity regarding the definition of dysbiosis, which is a widely used term in the field of probiotic research. Dysbiosis is primarily used to indicate an "abnormal" microbiome, that includes not only changes in microbial diversity, but also used to indicate the reduction of keystone taxa (chief microbial species that influence the composition and function of the microbial community), colonization by pathogens, and alterations in metabolic capacity, which contributes a functional aspect to the structural elements. Dysbiosis is hard to be specifically defined because the opposite term, eubiosis, which indicates a healthy microbiome under homeostasis, is a highly heterogeneous state. Interestingly, reports on the profiling of microbiomes among individuals have revealed unique inter-personal taxonomical patterns, which is even evident among twins. However, the existence of a stable core of functions and thus genes (the microbiome core) in an individual has been substantiated. The exact definition of what constitutes a "healthy" gut microbiota and the mechanism by which various strains of gut microbiota affect their host is still being developed. It has been very difficult to reach a consensus due to the variations observed in various studies, such as different strains, doses, and also the duration of the treatment.

6.2 Probiotics: Relevance in Management of AAD

Ever since the dawn of the concept of probiotics as proposed by Elie Metchnikoff (1907), the hypothesis that consumption of specific microorganisms could impart health benefits has fascinated the scientific community. The term "probiotics" itself first appeared in 1974 and has conceptually evolved with newer insights due to focussed research efforts to the current accepted definition, which is "live microorganisms that confer a health benefit when consumed in adequate amounts," as per the mandate provided by the World Health Organization in 2002 (Hill et al. 2014). In the current scenario, probiotics constitute a multi-billion dollar industry

projected to reach USD 65.87 billion by the year 2024 and are among the most popular food supplements consumed across the globe (Zion Market Research 2018). In order to impart health benefits, popular foods such as fermented dairy products (yogurt, cheese, etc.), ice cream, infant formulas, and nutrition bars been supplemented with probiotics. Probiotics have also been commercialized as lyophilized pills (Hoffmann et al. 2014).

Across the decades, the consumption of probiotics has received steady support by medical practitioners, specifically gastroenterologists. It is notable that despite the soaring popularity of probiotics among the medical fraternity as well as the consumers, insights from research efforts to elucidate the efficacy of probiotics for the management of various health conditions, can at times, point to ambiguous inferences. This is primarily due to the inter-species and even intra-strain variation that is observed among the probiotics that have been used in the clinical studies. Additionally, it is imperative to note that a probiotic strain would vary in its efficacy to impart specific health benefits among different population sub-groups (ethnic, dietary habits, age groups, etc.). Thus, microbiome and probiotic research has received an increased focus in recent years. Oral administration of probiotics provides a viable supply of beneficial bacteria for the management of gut health, enhancing nutrient absorption, and immunomodulation. Multiple in vivo studies as well as clinical trials have provided valuable insights regarding the efficacy of probiotics to effectively modulate the immune system. An open-labelled clinical study involved 18 healthy subjects, who were administered probiotic *Bacillus subtilis* HU58 capsules (2×10^9 CFU/Cap) once a day orally for 8 weeks. At the end of test period, it was observed that administration of probiotics was effective in reducing the levels of cytokines such as IL-6 and TNF-α by 45% and 55%, respectively.

Bacillus subtilis produces a variety of antimicrobial molecules such as bacteriocins, subtilin, and subtilosin and have also been extensively studied at genetic and physiological levels. Reports have demonstrated the efficacy of *B. subtilis* in the effective management of traveller's diarrhea caused by *Citrobacter rodentium* in murine model [14]. *B. subtilis* has been demonstrated to improve clinical, microbiological, and immunomodulatory efficacy in case of acute infectious diarrhea in young children. In poultry, *B. subtilis* has been shown be effective against pathogenic infections caused by *Salmonella enterica*, *Clostridium perfringens,* and *E. coli*. In vitro studies have also shown its potential efficacy against *Helicobacter pylori*. The HU58 strain of *Bacillus subtilis*, isolated from healthy human volunteers has been studied extensively and has been proven to be more stable in the highly acidic gastric environment. In addition, it can grow and sporulate in the anaerobic GI tract with high sporulation efficiency and enhance gut colonization through the development of biofilms and production of surfactant molecules that enhance adhesion to the gut mucosa (Mehta et al. 2020).

It is imperative to note that, since 2001, multiple *B. subtilis* strains, which were already commercially exploited as probiotics and also in other industries, were taxonomically reclassified as *B. clausii* (Khatri et al. 2019). Currently, *B. clausii* in commercially marketed as a probiotic formulation (Trade name: Enterogermina®),

which is composed of four strains of *B. clausii*, based on their resistance to various antibiotics O/C (chloramphenicol), N/R (novobiocin/rifampicin), SIN (neomycin/streptomycin), and T (tetracycline). These four strains have been established as being derived from *B. subtilis* ATCC 9799, which originally was resistant to penicillin (Mazza et al. 1992). The intrinsic resistant to antibiotics is advantageous to restoring healthy gut function, especially in cases where probiotics are administered in combination with antibiotics (Varankovich et al. 2015). *B. clausii* O/C strain is proven to inhibit the cytotoxic effects induced by toxins secreted by *C. difficile* and *B. cereus*. The specific mechanism of action of strains of *B. clausii* is yet to be fully understood, but research insights have provided information about the secretion of specific proteins which play a vital role in colonization of human GIT, immunomodulation, etc. (Lopetuso et al. 2016; Pradhan et al. 2016). The sporeforming probiotic strains have important advantage over non-sporeforming probiotic strains in terms of commercial application due to enhanced resistance to heat, desiccation, and exposure to chemicals, which are encountered during product development.

The genetic difference among probiotic bacterial strains is significant, and leads to the inability to extrapolate the function of one strain to another. This unique quality among bacterial strains, termed as strain specificity, and is well established with research studies, such as carried out by Douillard et al. (2013), where 100 strains of probiotic *Lactobacillus rhamnosus*, originally isolated from human as well as dairy sources, were proven to possess remarkable different probiotic attributes (viz. tolerance to bile acids, carbohydrate metabolism, and the ability to produce mucus-binding pili). The results provide more clarity to the concept that taxonomic profiling is not sufficient as a measure of functional efficacy of the strain, and the need for probiotic strains to be selected based on the evidence available to prove their functional attributes as opposed to total reliance on the popularity of the name of their particular species.

The symbiotic component of the intricate relationship between humans and their microbiome has garnered increased research focus during the past decade. The major component of the microbial population is located in the gastrointestinal system, and is known as gut microbiome, which includes their collective genomes (Marchesi and Ravel 2015). The results from the extensive research highlight the pivotal role played by the gut microbiome to contribute to the homeostasis of the human host, in health and also in the onset and development of disease. Beneficial attributes of gut microbiota have been classically linked with improved gastrointestinal function and healthy immune response (Thaiss et al. 2016; Azcarate-Peril et al. 2017; Fetissov 2017; van de Wouw et al. 2017). Also, important adverse effects were reported that range across a variety of health conditions, with the primary cause pointing to an altering of the gut microbiota composition and the subsequent interaction of metabolites that are the by-product of bacterial metabolism of the dietary components (Miele et al. 2015; Budden et al. 2017; Yu and Schwabe 2017; Schirmer et al. 2018). Consequently, there has been a heightened interest about probiotic products for their ability to promote wholesome health and also for

effective therapeutic modulators of the onset and development of a variety of diseases.

Despite compelling evidence for the administration of probiotics for their efficacy in the effective management of a variety of health issues ranging from improved digestion to neurological health, relatively few probiotic strains are available in the market and probiotics are yet to become part of routine clinical practice (Van den Nieuwboer et al. 2016). In addition, commercially available probiotic strains are claimed to provide a wide variety of health benefits spanning multiple health conditions without being substantiated using standardized study models (Day et al. 2019).

In order to counter it, regulatory agencies of various countries have developed their own framework to protect the interests of the consumers with respect to consumption of probiotic foods. The EFSA (European Food Safety Authority) has provided three critical regulatory parameters that need compliance prior to making a health claim. As a consequence, many health benefits claimed for probiotics have been rejected, and have resulted in the restriction of the use of the term "probiotic," as popular consumer sentiment implies that the very use of the term confers a health benefit. The regulatory issues discussed above mandate necessitate the demonstrated evidence of the health benefit (i.e., proof that the biomarker under investigation contributes to the claimed health benefit) and the capability to apply the same to the general population. Health claims about probiotics need to have specific details and general, vague claims involving phrases like "strengthens the immune system" are considered insufficient. Therefore, research studies have been focussed towards establishing the impact of probiotics on key biomarkers. It is important to note that, an established set of biomarkers with wide approval has not been finalized, which are well-correlated and validated by clinical inferences. As per requirements of EFSA, any health claims need to be clearly demonstrated using a minimum of two randomized, placebo-controlled clinical studies, with a scientifically validated mechanism of action including cause and effects of the probiotic efficacy (Rijkers et al. 2011).

In this perspective, poor study design, paucity of consistent research funding, and a lacunae in the understanding of the key mechanisms of action has resulted in "pilotitis"—where small studies without a specific focus have been unable to generate a robust evidence base, which is essential to obtain regulatory approval (Van den Nieuwboer et al. 2016). For example, in a recent systematic review with special emphasis on randomized controlled trials (RCTs), which deals with evaluating the efficacy of probiotic strains in the effective management of irritable bowel syndrome (IBS). Among the various RCTs (35 nos.) that were considered for the review, only three RCTs had a minimum of 100 participants, approximately and about 75% were pilot studies (McKenzie et al. 2016). Therefore, the need of the hour is well designed Phase III clinical trials to prove the efficacy of probiotics which is essential to provide the evidence base in order to generate confidence among health practitioners regarding the efficacy of probiotics.

6.3 Diversity of Gut Microbiota and Dysbiosis

The Human Microbiome Project (HMP1), a pioneering project, involving 250 healthy volunteers, under the aegis of National Institute of Health, concluded that the human microbiome is composed of 3500–35,000 species, which was based on operational taxonomic units (Morgan et al. 2013). The total count of bacteria harbored by the human body has been estimated to be hundred trillion (10^{13}), a value close to the total count of cells present in the human body (Sender et al. 2016). Thus, the human body accommodates a rich, highly diverse and unique microbial population. It is pertinent to note that there is significant variation in the diversity of microflora present in different body sites, e.g. the oral cavity and the colon have the greatest number of bacterial cells, with the least quantity of microflora observed in the vaginal region (Morgan et al. 2013). Interestingly, superior diversity of microflora in the gastrointestinal tract has been reported in individuals from primal hunter and gatherer populations, viz. *Hadza* from Tanzania (Turroni et al. 2016) and the *Yanomami* in Brazil (Clemente et al. 2015), which are potential indicators of the composition of our ancestral gut microbiota. These isolated and often dwindling ethnic communities display evidence for robust health contrary to the urbanized Westernized populations which are characterized by colonization by far less diverse microbiota (Moeller 2017). Low diversity of the intestinal microbiome is linked with a range of serious health issues, viz. Irritable Bowel Syndrome (IBS), Crohn's disease, colorectal cancer and also in obesity (Mosca et al. 2016). However, high diversity of microflora does not directly correlate with a healthy microbiome across various sites, as there are notable exceptions such as the vaginal region, where it is characteristic of bacterial vaginosis (Charbonneau et al. 2016), which is the most widespread cause of vaginitis.

Diversity is a key component in definitions of dysbiosis, a popular term referring to an"abnormal" microbiome. Microbiome profiling studies have recognized the presence of unique interpersonal patterns, which is evident even among the twins. Despite the unique features, the presence of an enduring core of specific functions and their corresponding genes has been established. The essential components of a 'healthy' gut microbiota and the mechanism of action regarding the impact of various species of gut microbiota and their interaction with the host is yet to be fully elucidated. This is further proving to be difficult due to the presence of variations among the strain, dose, and duration of treatment among different studies which hinder direct comparison and reaching a general consensus (Permpalung et al. 2016).

It has been well recognized that critical illness, particularly in hospitalized condition, has been linked with imbalance of gut microbiota, potentially causing increased susceptibility to contracting infection, which can lead to and ultimately, organ failure. During this scenario, the risk of developing antibiotic-related gut microbial dysbiosis, which also includes diarrhea, underscores the importance of rational administration of antibiotics and other antimicrobial agents (Appel-da-Silva et al. 2017).

6.4 *Clostridium difficile* Infection: Mechanism of Action

The key pathological conditions associated with *Clostridium difficile* infection (CDI) are primarily correlated with the activity of two toxins, TcdA and TcdB (Chandrasekaran and Lacy 2017). In *C. difficile* chromosome, the genes responsible for coding both TcdA and TcdB, in addition to the three additional toxins (Tcd C, TcdE, and TcdF) are found within the PaLoc (pathogenicity Locus), which has a size of 19.6 Kb (kilobase) (Kuehne et al. 2010). In terms of molecular nature, TcdA and TcdB share 49% homology in their amino acid composition (Navaneethan et al. 2010). The C-terminal domain of both the toxins binds to the intestinal epithelial cells, whereas the N-terminal domain is responsible for cytotoxic activity. The entry of the two toxins in to the epithelial cells is facilitated by the transmembrane domain. The gene products for the accessory toxins, TcdD upregulates transcription of the toxin, whereas TcdC, is a repressor for the genes coding for the toxin (Waligora et al. 2001). The gene product of TcdE is responsible for cell wall lysis and facilitating the release of TcdA and TcdB in to the intestinal lumen (Dicks et al. 2019).

Despite the fact that the respective roles played by these toxins in the pathogenesis have not been fully understood, various studies have provided insight regarding the synergistic action of the toxins. Other reports highlight the fact that many clinical isolates are unable to produce TcdA, supporting suggestions that TcdA is not essential for pathogenesis (Farrow et al. 2020). These clinical observations also concur with the in vivo studies, viz. infection studies using mouse and hamster models employing mutant strains for isogenic toxin have indicated that TcdB in itself is sufficient for the development of significant steps of the pathogenesis, including the key symptoms associated with the disease. In contrast, studies involving strains having the capability for the production of only TcdA (TcdA+TcdB-) have been either non-pathogenic or highly attenuated. The results of the studies provide insight into the significant development disease in case of the strains that are capable of producing TcdB (TcdA-TcdB+) and thereby clearly point to the central role played by TcdB in pathogenesis (Carter et al. 2015; Lyras et al. 2009). This is further substantiated by studies in gnotobiotic piglet model where an antibody against TcdB conferred protection against CDI (gastrointestinal and systemic types (Steele et al. 2013). This has also been further confirmed in clinical trials, where treatment with antibodies against TcdB was able to effectively reduce the incidence of relapse of CDI (Wilcox et al. 2017).

In terms of the enzymatic nature of the toxins, both the toxins (TcdA and TcdB) are homologous glucosyltransferases which can effectively alter and ultimately inactivate Rho family GTPases that are present in the host cell. The activity of these glucosyltransferases have been directly associated with disruption of the actin components of the cytoskeleton and eventually causes cytopathic changes such as disruption of the tight junctions. The disruption of tight junctions triggers the immune system and triggers the synthesis of pro-inflammatory cytokines (Pruitt and Lacy 2012). Additionally, along with these cytopathic changes, the toxin (TcdB) is an effective cytotoxin that eventually leads to necrosis of the affected host cells and tissue (Chumbler et al. 2012). The glucosyltransferase activity of the toxin is not

linked with the necrotic response. The necrotic effects of the toxin (TcdB) are due to the induction of the assembly of relevant NOX complex (NADPH oxidase) and the concurrent secretion of ROS (reactive oxygen species) (Farrow et al. 2013), which at elevated levels can lead to damage of mitochondria, peroxidation of lipids, and oxidation of proteins. Cell and tissue-based studies have demonstrated that the necrotic effects is unique to TcdB and occurs at certain concentrations (100 pM) (Chumbler et al. 2016). As the development of necrotic lesions are characteristic feature of colitis caused by *C. difficile*, and levels of the toxins are directly linked with the severity of the disease. It has been hypothesized that these mechanisms of action of the toxin (TcdB) produced by *C. difficile* are responsible for the onset and development of the characteristic symptoms of the disease (Farrow et al. 2020).

Antibiotic-associated diarrhea (AAD) is characterized by the imbalance in the homeostasis of the gut microbiota, and particularly, decreased concentrations of secondary metabolites [viz. short chain fatty acid (SCFA)] in the intestinal lumen, and the concurrent increase in the presence of carbohydrates in the intestinal lumen and biliary acids in the colon, along with impaired water absorption, and eventually causing diarrhea. As discussed previously, probiotics present a viable solution in the management of AAD in numerous clinical trials by modulating the gut microbiota, influencing the metabolism of bile salts and nutrients, inducing the action of epithelial solute transporters, augmenting the function of the intestinal barrier, and imparting positive effects leading to the effective modulation of the immune system.

In a four-week clinical study involving patients treated for CDI, oral administration of probiotics [consortium of probiotic strains (4 nos.) of *Lactobacillus* and *Bifidobacterium* in combination with antibiotics showed significant improvement with respect to reducing the duration of *C. difficile* diarrhea (Barker et al. 2017). Microbiological analysis of the fecal contents revealed that patients who were administered with probiotics contained reduced levels of Verrucomicrobiaceae in the gut, in comparison with placebo-treated groups (De Wolfe et al. 2018). Although other variations with observed with administration of the probiotic consortium, in microbiological composition of the gut, the reduced levels of the presence of members of Verrucomicrobiaceae were also consistent with the direct association of the susceptibility of the family of the patient to developing infection by *C. difficile* (Bassis et al. 2014). In case of a clinical study carried out subsequent to antibiotic therapy for the treatment of infection by *H. pylori*, it was observed that in case of patients taking a probiotic consortium (multiple strains of *Bacillus subtilis* and *Enterococcus faecium)* there was a reduction in the changes to fecal microbial composition due to antibiotic therapy changes in comparison with placebo-treated group (Wu et al. 2019). It is notable that other clinical studies have reported similar beneficial effects of probiotic administration during the antibiotic treatment period for management of *H. pylori* infection (Mekonnen et al. 2020).

The major global medical regulatory bodies, viz. the European Food Safety Authority (Rijkers et al. 2011) and the Food and Drug Administration (USA) (Saldanha 2008) have not yet provided approval for the use of any probiotic formulation for administration as a therapeutic agent. Subsequently, the probiotics are being marketed as dietary supplements with importance focussed mainly on the

safety, ability to survive the passage through the gastrointestinal tract, no adverse impact on the organoleptic attributes of the carrier product, instead of their unequivocal health-promoting effects. Therefore, the current scenario demands improved scientific proof of the key health benefits and adverse effects of the administration of probiotics (Sniffen et al. 2018).

The health benefits attributed to the consumption of probiotics in humans has received extensive research by scientific community as well as the food and pharmaceutical industry for decades. As a result, a myriad of health claims has been suggested encompassing both therapeutic and prophylactic health approaches, such as management of acute, ADI & CDI, IBS, IBD (Inflammatory bowel disease) and decreased risk of sepsis (late-onset type) and necrotizing colitis in neonatal subjects (Suez et al. 2019). Other health claims include, management of *Helicobacter pylori* infection, respiratory tract infections, neurological health (alleviation of depression, mood swings, etc.), and decreased cardiovascular risk factors linked with the cardiometabolic syndrome. It should be noted that in spite of data from many clinical studies positively affirming the health benefits mentioned above, comply with sound methodology and scientific validation (Gao et al. 2010; Panigrahi et al. 2017) for the major portion of these health conditions, there are also clinical studies of equal scientific validation that have featured contrasting negative results, thereby contributing to the development of rather ambiguous and inconclusive scientific ambience.

C. difficile flourishes in the gut mucosa in situations where colonization resistance due to microbial homeostasis is adversely affected (viz. due to antibiotic treatment among inpatients).

Through the outcome of focussed research in recent years, it is evident from several meta-analyses that administration of probiotics provides an augmentative positive effects for orally administered probiotics, both prophylactically and regarding the effective management of associated morbidity (Goldenberg et al. 2017), especially when the probiotics are administered close to antibiotic exposure (Shen et al. 2017). Additionally, subsequent follow-up of the meta-analysis involving 8672 cases involving various probiotic strains, age groups, dosage and duration of administration provided insights in to the ability to impart reasonable prophylactic effect in patients undergoing antibiotic therapy. In contrast, the results showed the presence of considerable heterogeneity between the clinical trials. Furthermore, post hoc analysis of the results failed to reveal significant beneficial impact of administration of probiotics on the protection of CDAD during clinical studies with subjects having low to medium risk of CDAD (Goldenberg et al. 2017). Similar conclusions have also been revealed with respect to other probiotic strains. For example, one meta-analysis showed that among different probiotic strains, only *Saccharomyces boulardii* was efficient against *C. difficile*. In contrast, other meta-analysis specially related to *S. boulardii* concluded that the strain was beneficial at decreasing the susceptibility to develop CDAD in children but not among adult groups (Szajewska et al. 2016).

Detailed analysis of the individual clinical studies that formed the basis for these meta-analyses has elucidated the incidence of *C. difficile* infection during the duration of the trial was non-existent or observed to be predominantly decrease in studies among the placebo and treatment groups. Also, majority of the studies that were considered for the meta-analyses did not provide clear evidence regarding the efficacy of various strains against infection by *C. difficile* or CDAD. This conclusion may be accounted for by the insufficient power of these clinical studies to provide clear evidence regarding the decreased incidence of infection caused by *C. difficile*. In contrast, among a couple of random controlled trials (RCTs) where special emphasis was directed on studying population groups with a high observed rates of *C. difficile* infection. The RCTs also featured one of the largest clinical studies of probiotic administration for the particular indication, and the results revealed no significant difference among the placebo and treatment groups (Allen et al. 2013; Szajewska et al. 2016). Therefore, the clinical evidence for prophylactic effects of the administration of probiotics to tackle CDAD is primarily supported by only minority of studies (Suez et al. 2019).

Research findings have also provided insight regarding the role of probiotic strains on the expression levels of specific immune-related genes, activity of key inflammatory pathway, and levels of important immune markers, which include modulation of NF-κB (intestinal epithelial cell), mitogen-activated protein kinase (MAPK), Akt [i.e. phosphoinositide 3-kinase PI3K], peroxisome proliferator-activated receptor-γ, CRP, interleukin (IL)-6, IL-8, tumor necrosis factor (TNF)-α, IL-1β and interferon-γ (IFN-γ). Probiotics have been purported to act through multiple mechanisms that are primarily driven by interaction with the right target cells (Thomas and Versalovic 2010). Interestingly, certain studies involving viable and dead probiotic bacteria have shown a variance about their effect on the expression of genes, providing insight regarding the importance of the surface of the bacterial cell as well as the secreted molecules on the intestinal transcriptome (Van Baarlen et al. 2009). In addition, studies have reported on the ability for immunomodulation in the host by probiotics which is evidenced through the TLR2 (toll-like receptor 2)-dependent stimulation of the secretion of TNF-α secretion in conjunction with lipoteichoic acid (LTA), observed in the case of *Lactobacillus* sp. (Matsuguchi et al. 2003), contact-dependent secretion of IL-10 mediated by *B. longum* (Medina et al. 2007), stimulation of TNF-α response by sortase-dependent pili in *Bifidobacterium* (Turroni et al. 2013), cell surface exopolysaccharide (sEPS) of *B. longum* 36,524 able to modulate secretion of pro-inflammatory cytokines and response of T-helper cell 17 (TH17) in colon as well as in the lungs (Schiavi et al. 2016). Also, in in vitro models, immunostimulatory cell surface appendages (SpaCBA), in *Lactobacillus rhamnosus* GG have been shown to be capable of mediating adhesion to mucus present in the human intestine as well as TLR2-dependant immunomodulation of the secretion of cytokines (TNF-α, IL-6, IL-10, and IL-12) (von Ossowski et al. 2013). Using in vivo models, LGG has also been shown to be effective in elevated production of ROS (reactive oxygen species) and the subsequent inhibition of that is induced by TNF-α

via adhesion to the intestinal epithelium mediated by SpaC appendages (Ardita et al. 2014) (Table 6.1).

In vivo studies (mice model) have also shown that cell wall peptidoglycan from *L. salivarius* Ls33 is effective in prevention of colitis (chemically induced) through the association with nucleotide-binding oligomerization domain-containing protein 2(NOD2)–IL-10-dependent mechanisms (Fernandez et al. 2011). However, the same beneficial effects were not reported in *L. acidophilus* NCFM. Similar studies have provided insight regarding the ability of *L. acidophilus* L-92 to bind to M cells (microfold cells), and providing immunomodulation via its surface layer protein A (SlpA); other examples include the ability of *B. infantis* 35,624 to induce TLR2-depended T-regulatory cells in clinical studies, and also inducing the secretion of IgA by *B. animalis lactis*Bb-12 (Yanagihara et al. 2017).

It is noteworthy that in majority of the studies quoted above, there is a need for the occurrence of physical contact(proximity) between probiotic bacteria and the host cells for the potential induction of both pro- and anti-inflammatory responses, which provides special relevance about the mode of administration of probiotics. Therefore, further clinical studies are required to establish their beneficial outcomes in humans where these probiotic strains are capable of successful colonization of the gut mucosa. Additionally, understanding the molecular mechanisms of probiotic action in the gut is critical for effective use of the existing probiotic strains with specific focus on the recommended dosage, frequency and the total duration of administration, the significance of employing a consortium involving multiple probiotic strains, and the optimal protocols and the components for the manufacturing of the probiotic products. In addition, understanding the molecular mechanisms of action can also help in select in the next-generation probiotics.

6.5 Short Chain Fatty Acid (SCFA) Metabolism and AAD

Among the research studies on probiotic efficacy, there is good consensus on the hypothesis that the modification of the metabolism of nutrients caused by the remodelling of the gut microbiome that is exposed to antibiotic therapy can significantly alter the intestinal metabolome. The chief factor that contributes to the metabolite alteration in the lumen is the decrease in the levels of short chain fatty acids (SCFA) (Binder, 2010; Theriot et al. 2014). SCFAs (viz. acetate, butyrate, and propionate) are the principal metabolic by-products of bacterial metabolism of carbohydrate in GIT and take up to 10% of the daily energy requirement in humans (van der Beek et al. 2017). A reduction in the biosynthesis of SCFAs in the gut might lead to the development of AAD because they promote absorption of water and sodium chloride.

SCFAs are efficiently taken up in the large intestine and stimulate fluid absorption (sodium-dependent) through a cyclic AMP-independent mechanism with exchanges of Sodium-Hydrogen, SCFA-Chloride, and SCFA-bicarbonate moieties (Fig. 6.1).

Antibiotic therapy also disrupts the GI tract microbiota via loss of homeostasis, which causes amplified colonization by opportunistic pathogens, the build-up of

Table 6.1 Molecular mechanisms of the efficacy of probiotic strains in the management of antibiotic-associated diarrhea (AAD) in humans and in vivo models (Mekonnen et al. 2020)

Antibiotic	Pathogen treated	Responses to probiotics			
		Probiotic strain employed & study parameters	Gut microbiota & metabolome	Expression of intestinal genes and proteins	Physiological effects
Vancomycin or metronidazole	*C.difficile*	*L. acidophilus* NCFM, *L. paracasei* Lpc-37, *B. lactis* Bi-07, and *B. lactis* B1–04,	↓ Verrucomicrobiaceae ↓ Bacteriodetes	ND	↓ Total duration and diarrhea No change in *C.difficile* recurrence
Clarithromycin, amoxycillin, and esomeprazole	*H. pylori*	*B. subtilis and Streptococcus faecium*	↑ Alpha diversity of Bacteria ↓ Alterations to the composition of bacterial community ↓ Genes responsible for the metabolism of amino acids and carbohydrates	ND	ND, small sample size
Amoxicillin-clavulanate; Healthy volunteers		*S. boulardii* CNCM I-745; concurrent with antibiotics	↓ *E. coli* ↓ *Parabacteroides* and *Ralstonia* ↑Fecal secondary bile acids	ND	↓ AAD scores
Ciprofloxacin & metronidazole; Healthy volunteers		Consortium of 11 strain composed of species of *Lactobacillus, Bifidobacterium, Lactococcus and Streptococcus*, post-antibiotic treatment	↓ Recovery of the indigenous microbiome (fecal and mucosal), comprising: ↓ Alpha diversity of bacteria ↓ Bacterial load ↓ Clostridiales	↑ Ileum REG3G and colon IL-1B transcripts	ND

(continued)

Table 6.1 (continued)

Antibiotic	Pathogen treated	Responses to probiotics			
		Probiotic strain employed & study parameters	Gut microbiota & metabolome	Expression of intestinal genes and proteins	Physiological effects
Metronidazole, *C.difficile* treatment		*L. plantarum* 299V; concurrent with antibiotic therapy	No significant change in SCFA	ND	Marginal improvement in recurrence of clinical symptoms (small sample size)
In vivo models					
Piglet					
Aureomycin		*Bacillus amyloliquefaciens* SC06; concurrent with antibiotics	ND	↑JejunmIL-6, MyD88, NOD-1, TLR-4, and TNF alpha transcripts ↑TNF alpha in reum ↓ TNF alpha in liver, IFN-gamma, IL-6 & IL-10	↑Intestinal villus height ↓ Crypt depth ↓ Gut permeability
Rat					
Clindamycin, ampicillin, streptomycin		*B. fragilis* ZY-312 in different daily doses (10^7–10^9 CFU) and/or a mixture of *B. longum*, *L. acidophilus* and *E. faecalis* post-antibiotic treatment	No change in bacterial alpha diversity ↑*Akkermansia* ↓ *Escherichia*	↑Colon Aqp1, Aqp3, and Aqp 8 transcripts ↑ Mucin 2 transcripts, Colon ZO-1, occludin ↑ KI-67 positive cells with the highest dose of *B. fragilis* ZY-312	↓ Fecal water ↓ Fecal consistency score with the highest dose of *B. fragilis* ZY-312
Mice					
Clindamycin (subcutaneous)		*L. paracasei* CNCM I-3689; concurrent with antibiotics	↓ Vancomycin-resistant enterococci ↑Bacteriodetes	↑Ki-67 & PCNA-positive cells	ND

and *E. faecalis* V583					
Metronidazole, neomycin & vancomycin		*L. rhamnosus* GG; concurrent with antibiotics	ND	↑GPR109a, SLC5A8, SLC26A3, AQP4 &NHE3 transcripts	No effect on body weight of mice.
Cefixime		Two strain mixtures with *L. plantarum, L. casei, L. rhamnosus* and *L. helveticus,* and fructooligosaccharides; post-antibiotic treatment	↑Recovery of composition of microbiota: ↑Firmicutes ↓ Bacteriodetes ↑Proteobacteria ↑SCFA	↓ Serum CRP, C3 & IgG	↓ Cecum size ↓ Inflammatory filtrate
Ampicillin		Separate groups given *L. casei*, CGMCC12435 (LacC), *L. plantarum* CGMCC 12436 (LacP), or *L. rhamnosus* GG (LacG); post-antibiotic treatment	↑ Recovery of microbiota composition ↑Alpha diversity ↑ Bacteriodetes ↓ Proteobacteria (LacC & LacP) ↑SCFA (LacC)	↑Ileum ZO-1, occludin (LacC), Claudin −1 (LacP) transcripts ↓ NF-kB (LacC, LAcP) ↓ IL-1b, IFN-gamma (LacC) ↓ Reg-3gamma (LacG) ↓ sIgA (LacC, LacG) in the colon	↓ Gut permeability ↓ Serum endotoxin ↓ Diamine oxidase

Abbreviations used: ND: Not Detected, *C3: Complement 3, sIgA: secretory immunoglobulin A, aqp: aquaporin, GPR109A: G-protein-coupled receptor 109A, SLC5A8: Solute Carrier Family 5 Member 8, SLC26A3: Solute Carrier Family 26 Member 3, and NHE3: Na+/H+ exchanger 3. For reference (Suez* et al. 2018*), a multi-strain mixture was used as follows: L. acidophilus ATCC4356, L. rhamnosus (strain designation not provided), L. caseiATCC393, L. paracasei ATCC BAA-52, L. plantarum ATCC8014, B. longum subsp infantis ATCC15697, B. bifidum ATCC29521, B. breve ATCC15700,B. longum subsp. longum ATCC15707, Lactococcus lactis (strain designation not provided), Streptococcus thermophilus ATCC BAA-491. For reference (Shi* et al. 2018*), a multi-strain mixture was used as follows: L. plantarum including CCFM4, CCFM10, CCFM595, CCFM602, & CCFM605; L. casei CCFM5, CCFM30, CCFM236, CCFM2710, and CCFM2711; L. rhamnosus LGG, CCFM237, CCFM311, CCFM319 and CCFM492; and L. helveticus CCFM6, CCFM672 and CCFM673*

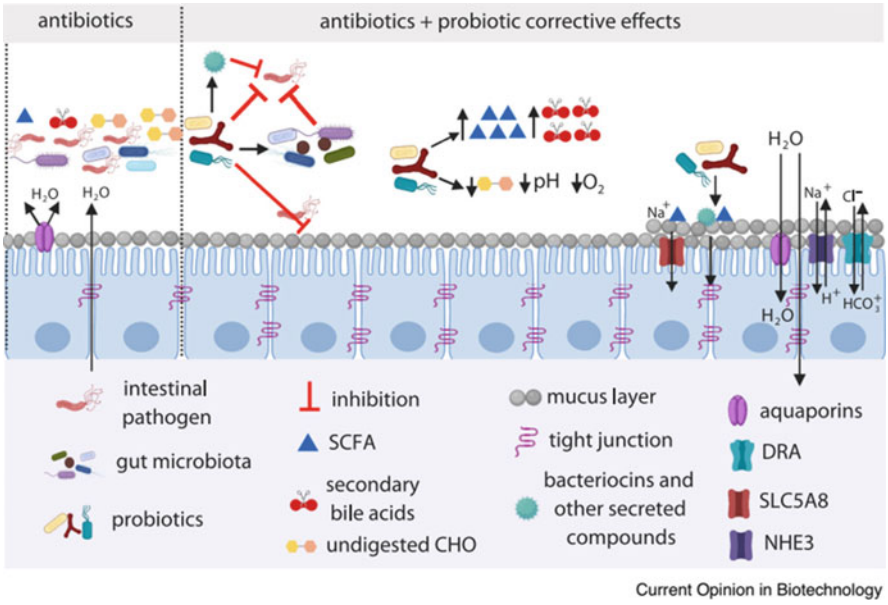

Fig. 6.1 A schematic model of the potential molecular mechanisms of probiotic action towards prophylaxis of AAD (Mekonnen et al. 2020)

unutilized carbohydrates and decreased concentration of secondary bile acids and SCFAs. Probiotics could effectively tackle these changes by direct antagonism towards pathogens or through inducing changes in the gut microbiota composition, chiefly by the elevated production of SCFAs, production of secreted metabolites viz. bacteriocins, reduction of luminal pH and oxygen concentrations. Probiotic strains may also influence the composition of biliary acids in addition to positive interaction with intestinal epithelium and the immune system of the host ultimately leading to enhanced gut barrier function besides the effective regulation of water and solute transport.

In mice model, *L. rhamnosus* GG was effective with an efficiency matching tributyrin (a derivative of butyrate) in providing prophylactic effects in case of intestinal injury induced by antibiotic therapy and reductions the levels of SCFA receptor (GPR109a) and transporters(SLC5A8) (Cresci et al. 2013). Since *Lactobacillus* spp. do not have the pathways that are needed for the production of butyrate, the increased luminal butyrate levels of the administration of *L. rhamnosus* GG has been hypothesized to be a case of cross-feeding with other components of the gut microbiota, induced by probiotics. Clinical trials have also provided evidence of the positive impact of probiotics regarding the intestinal SCFA levels. *L. plantarum* 299V has been proven to be effective in arresting a decrease in SCFA during treatment with metronidazole (Wullt et al. 2007).

In cell line models, metabolites secreted by *L. acidophilus* ATCC4537 were able to prevent the inhibition of the uptake of butyrate by Caco-2 cells mediated by

enteropathogenic *E. coli* through the inhibition of MCT1 (monocarboxylate transporter isoform 1) endocytosis. Imperatively, the same capability has not been observed in case of heat-killed *L. acidophilus* or by viable cells of three other *Lactobacillus* strains tested (Kumar et al. 2015). Thus, there is a strong indication of strain-specific factors being responsible for the observed effects.

Probiotics might also directly influence the SCFA levels in the intestinal lumen by the production of organic acids (viz. acetate, lactate, etc.) and by encouraging the presence of SCFA-producing gut bacteria. The production of SCFA (viz. acetate) by *Bifidobacterium* spp. in GIT has been proven to effectively decrease the risk for infection by enteropathogenic *E. coli* (Fukuda et al. 2011). Probiotic metabolism and production of organic acids (e.g. lactate and acetate) in situ could lower luminal pH and oxygen levels in addition to acting as substrates used for the synthesis of butyrate and propionate by microbial components of the gut microbiota (Louis and Flint 2017). In addition, the colonization of probiotics in GIT might decrease the levels of undigested carbohydrates, leading to a reduced risk of developing diarrhea caused by osmogradient modifications (Binder 2010).

6.6 Modulation of the Secretion of Electrolyte Secretion and Absorption Efficacy

The inefficient absorption as well as the active secretion of electrolytes (solutes) by the intestinal epithelial cells contribute to the clinical manifestation of watery diarrhea. Electrolyte concentrations in GIT are regulated by multiple baso-lateral and apical channels along with transporters regulating the transport of chloride (Cl-) as well as active transport of sodium (Na+) through the epithelial barrier with parallel absorption of chloride (Cl–) or bicarbonate (HCO3–)ions (Camilleri et al. 2017; Thiagarajah et al. 2015). In case of in vivo mice model, *B. subtilis* CU1 (CNCM I2745) was shown to stimulate the expression of elevated levels of NHE3 (epithelial Na+/H+ exchanger-3 protein), which enhances fluid absorption, and reduced levels of cystic fibrosis transmembrane conductance regulator (CFTR), a protein component which plays a pivotal role in the secretion of chloride ions (Urdaci et al. 2018). The similar effects were not reported in *L. plantarum* CNCM I-4547.

In another in vivo study (mice model), *L. acidophilus* ATCC4357 was able to prevent diarrhea by effectively tackling the inhibition of NHE3 protein caused by infection of *Citrobacter rodentium*. Additionally, the Cl/HCO3exchanger protein (DRA) was also reported to retain its activity subsequent to treatment with *L. acidophilus* (Kumar et al. 2016). Analogous results have been demonstrated involving other studies, such as Bacteroides fragilis ZY-312 (Zhang et al. 2018) & *L. rhamnosus* GG (Cresci et al. 2013), which resulted in the upregulation in the expression of genes specifically coding for membrane proteins that act as aquaporin water channels. Therefore, the alterations mediated by probiotics with respect to the various electrolyte transporters present in the GIT might represent an effective mechanism for the efficient management of AAD.

Besides SCFAs, other metabolites secreted by probiotics might also confer similar beneficial effects such as gassericin A, a bacteriocin produced by *L. gasseri* and *L. frumenti*. In vitro tests have shown that gassericin A is effective at increasing intestinal fluid absorption by inducing higher levels of cellular cyclic nucleotide observed in epithelial cells through mechanisms that involves binding to the membrane protein Keratin 19 (KRT19) and effectively activating mTOR (mechanistic Target of Rapamycin) phosphodiesterase activity. In vivo studies using piglets have also demonstrated the efficacy of gassericin A producing *L. gasseri* in prevention of diarrhea (Hu et al. 2018).

6.7 Increase in the Concentration of Secondary Bile Acids

In healthy humans, an estimated 95% of the luminal biliary acids are subject to reabsorption in distal region of ileum (Winston and Theriot 2020), with the remaining bile acids being subject to modification by intestinal bacteria and subsequently passively absorbed or excreted. Antibiotic therapy disrupts this balance and causes an increase in the levels of primary biliary acids present in the gut, which inhibit activity of epithelial ion transport proteins. It has also been shown that decrease in the microbially altered, secondary biliary acids elevates the susceptibility to develop infection by *C. difficile* (Buffie et al. 2015). Clinical studies have demonstrated the efficacy of *S. boulardii* CNCM I-745 to alter the biliary acid composition during antibiotic therapy among healthy volunteers. Higher quantities of primary biliary acids (cholic acids), and lower levels of secondary biliary acids have been reported in the fecal samples from subjects on treatment with antibiotics (amoxicillin-clavulanate). Administration of *S. boulardii* CNCM I-745 was also reported to reverse these changes in clinical studies (Kelly et al. 2019).

Deconjugation of bile acids by the secretion of bile salt hydrolases (BSH) by probiotics (viz. species of *Bifidobacterium*, *Lactobacillus,* and *Clostridium)* is already well established. BSH deconjugate biliary acids which can be additionally metabolized to secondary and tertiary biliary acids by the action of other components of gut microbiota (Winston and Theriot 2020).

In vivo studies (mice model) have demonstrated the efficacy of enhanced BSH activity in ameliorating the cardiometabolic impacts of high lipid diet (Joyce et al. 2014). Research insights have revealed that genes coding for BSH are selectively enriched among *Lactobacillus* spp. that are associated with vertebrates (O'Flaherty et al. 2018). Significantly, gut-associated BSH phylotype with the most effective enzymatic activity was primarily reported in *Lactobacillus* spp. and not other components of the human gut microbiome (Song et al. 2019).

6.8 Augmenting the Intestinal Barrier Function

The integrity of intestinal epithelial barrier has been showed to be very crucial for the pathologenesis of a variety of intestinal as well as other systemic diseases (König et al. 2016). As per in vivo studies carried out using rodent model, it has been shown that antibiotic therapy induces deficits in barrier function, also termed as a "leaky gut," but its severity varies based on the specific type of antibiotic that has been employed for the therapy (Tulstrup et al. 2015). In animal studies, particular strains of probiotics have been demonstrated to thwart these disruptions to the intestinal epithelium that are induced by antibiotics. For example, *Bacillus amyloliquefaciens* was reported to provide enhanced structural and functional attributes of the epithelium in piglets that were administered aureomycin (Du et al. 2018). Also, similar effects were observed in case of *Lactobacillus casei* CGMCC 12435 and a combination of *Lactobacillus* and *Bifidobacterium* strains in mice subjected to ampicillin treatment. The results were substantiated by the observation of enhanced transcripts for tight junction proteins (Shi et al. 2018). Administration of *B. fragilis* ZY-312 (10^9 CFU/day) was observed to increase ZO-1 as well as occluding, tight junction proteins, production of mucin, and cell markers for the proliferation of epithelial cells in the colon (Zhang et al. 2018). Additional research efforts are needed to expound the role of specific metabolites produced by probiotics that impact epithelial barrier function (Bron et al. 2017). Recent reports involving in vitro models have demonstrated that Plantaricin EF, a bacteriocin secreted by *L. plantarum* can effectively thwart pro-damage to the barrier integrity caused by the action of inflammatory cytokines. Additional extracellular proteins secreted by bacteria such as the outer membrane, pilus-associated protein synthesized by *Akkermansia mucinophilia* could also provide improvements to the effectiveness of the intestinal barrier (Plovier et al. 2017) (Fig. 6.1).

6.9 Modulation of Intestinal Immune Response

Antibiotic therapy has also been proven to adversely affect the homeostasis of the immune system of the host. In clinical studies, antibiotic therapy has been associated with impaired responses to vaccination, among subjects with reduced prevalent antibody titres (Hagan et al. 2019). In mice model, antibiotics have also been known to induce chronic, macrophage-dependent elevation of the inflammatory T-helper 1 (TH_1) and heightened susceptibility to certain infections (Scott et al. 2018).

Putative and proven probiotic strains of microorganisms are demonstrated to effectively protect against antibiotic-associated activation of inflammatory pathways in both in vivo models (mic and piglets) as well as in humans (Suez et al. 2018). These are supported by observed reductions in the quantities of C-reactive protein, complement proteins (C3), and antibodies (IgG) which indicate the efficacy of probiotics to limit the systemic effects of antibiotics. The reports concur with the strain-dependent immunomodulatory activities of probiotics among healthy subjects

and also individuals with immune system mediated chronic diseases (e.g. allergy, asthma) (Galdeano et al. 2019; Peters et al. 2019). Although additional research efforts are necessary to bring to light the role of specific components of the probiotic cell that directly alter the immune function during antibiotic therapy, recent reports have suggested that the exopolysaccharides produced by *Bifidobacterium* (Schiavi et al. 2016) and *Lactobacillus* S-layer proteins (Lightfoot et al. 2015) have immunomodulatory function. Thus, immunomodulation by probiotics during antibiotic therapy could be mediated by the secretion of multiple compounds.

Despite the large quantum of studies which indicate the efficacy of probiotics to effectively treat AAD, it is significant to note that very few studies have investigated the molecular mechanisms of their action. It is particularly to be noted that very few mechanistic studies using in vivo models and clinical trials have directly examined the most popular choice of probiotics for clinical trials, viz. *L. rhamnosus* GG or *S. boulardii* CNCM I-745. It has been hypothesized that the positive effects imparted by probiotic strains are multi-factorial and strongly dependent on the probiotic strain as well as the health status of the host (Goldenberg et al. 2015).

The presence of mechanistic overlap among probiotic strains (e.g. beneficial effects caused by the secretion of SCFAs), in addition to host–microbe interactions that are strain-specific in nature (e.g. beneficial effects due to secretion of key enzymes) have also been hypothesized (Hill et al. 2014). These beneficial effects need to be assessed in aptly regulated, multi-center clinical studies where responses of the intestinal and gut microbiota are analyzed and validated by supporting in vivo studies applying the same protocols.

Therefore, it is of paramount importance that the elucidation of the molecular mechanisms of action of probiotics in GIT is of chief importance for developing viable recommendations for current probiotic strains in specific areas (viz. recommended dose, frequency and total duration of the administration of probiotics). In addition, it also provides insight regarding the value of using a consortium comprising multiple strains of probiotics and the optimal protocols for its manufacturing and carrier delivery. It can also be applied for developing appropriate techniques for the selection of next-generation of probiotics with enhanced efficacy to facilitate the management of AAD.

6.10 Conclusion

The fascinating research insights summarized in the present chapter provide clear understanding of the holistic and comprehensive role played by a variety of probiotic strains in the effective management of AAD. The global incidence of AAD is only going to increase owing to the widespread and unscientific administration of broad-spectrum antibiotics as well as the rising trend of high stress, coupled with imbalanced diet and unhealthy lifestyle among the population. These changes are primarily due to rampant urbanization and the subsequent migration of rural population to the urban centers in India as well across the developing world. The overall scenario provides strong impetus to the addition of probiotics in the arsenal to

effectively tackle AAD. This would be further hastened with specific research efforts targeting the lacunae discussed in the present chapter especially the need for comprehensive, well-planned clinical studies to provide unequivocal evidence for the efficacy of specific probiotic strains, which would enable the authorized regulatory agencies to approve its administration to benefit the patients affected by AAD.

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7 Potential Correlation Between Homeostasis Control and Tumor Microenvironment Regulation of Probiotic as a Therapeutic Agent to Manage Gastrointestinal Cancer

Nabendu Debnath and Ashok Kumar Yadav

Abstract

Gastrointestinal (GI) cancer possesses a serious global public health problem. GI cancer refers to a group of cancer that affects various parts of digestive system that includes gastric cancer (GC), colorectal cancer (CRC), hepatocellular carcinoma (HCC), esophageal cancer (EC), and pancreatic cancer (PC). As compared to other cancer cases, GI cancer accounts 25% of all cancers and causes almost 9% of all cancer related deaths worldwide. The human intestinal microbiota interacts with host and influences a plethora of activities, such as metabolism, nutrient absorption, provides resistance against pathogenic microorganisms, and also plays an important role in the development of the gut immune system. Gut-microbiota is also capable of synthesizing some vital metabolites inside the gut such as short-chain fatty acids, essential vitamins, and microbiome dysbiosis, however, might lead to disruption of the homeostasis of the immune system and mucosal barrier functions. This leads to subsequent inflammation resulting in increased mucosal barrier permeability and a continuous state of inflammation. Oral administration of distinct probiotic strain as a functional food has been suggested to affect multiple processes in host and reduces cancer risk. Probiotics are mono or mixed cultures of live organisms that confer beneficial health effects to the host upon ingestion in adequate amounts. The readily available probiotic preparations present in the market are mainly based on lactic acid bacteria. Probiotics show anticarcinogenic effects by reducing activities of microbial enzymes, inducing several cytokines which ameliorate or prevent tumorigenesis through modulation of the host's cellular immune responses, binding potential to

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carcinogen, producing antitumorigenic or antimutagenic compounds in the colon, altering physiologic conditions (such as pH) in the colon, affecting the metabolic activity of intestinal microflora. A wealth of indirect evidence based largely on laboratory studies has indicated the possible positive effects of probiotic consumption on cancer suppression.

Keywords

Probiotics · Gastrointestinal cancer · Anti-cancer · Homeostasis · Gut immune system

7.1 Introduction

An adequate number of viable organisms that stimulate other beneficial microorganisms' growth and aid in health after being administered to host are known as probiotic microorganisms. According to the latest definition, probiotics are defined as "live microorganisms which when administered in adequate amount induce health benefits in host" (Hill et al. 2014). Those bacteria which form the lactic acid bacteria (LAB) group include *Lactobacillus* spp., *Bifidobacterium*, *Streptococcus thermophilus, Enterococcus, Escherichia coli,* and *Saccharomyces boulardii*. Probiotics render anticarcinogenic effects via vivid modifications either in molecules or various metabolic and other pathways.

The human intestinal tract harbors over 400 different bacterial species. The gut microflora and its distribution are modulated by physiological interactions, diet, and other related factors. Many of the bacteria could not survive in the acidic pH of gastric juice and only acid resistant bacteria thrive in stomach. In addition, throughout the small intestine a transition region of low and high population of bacteria can be observed and colon region harbors large quantity of bacteria instead. Human gut–microbiota plays an important role in gastrointestinal homeostasis and modulates various functions of the same. Studies have also co-related gut-dysbiosis with various types of diseases including gastrointestinal (GI) cancers. GI cancer accounts for 25% among all kinds of cancer burden worldwide with increasing trend (Hill et al. 2014). GI cancer is malignant condition of gastrointestinal tract and organs related to it like esophagus, stomach, biliary system, pancreas, small intestine, large intestine, rectum, and anus. Surgery is the main means of treatment of GI cancer at present (Van Cutsem et al. 2016). Cardinal features of GI cancer include loss of certain functional events, inactivation of tumor suppressors and apoptotic pathways, epigenetic alterations (DNA methylation pattern), and aberrant gene expression and silencing (Lynch and Rustgi 2012). Majority of gastric cancer mainly occurs in developing countries and females are more commonly affected than males (Fig. 7.1).

Obesity and type 2 diabetes are mostly correlated with the occurrence of various kinds of GI cancers (Lynch and Rustgi 2012; Petruzzelli and Wagner 2019). Specific preventive strategies such as using functional foods containing probiotics could provide opportunities to mitigate obesity and metabolic health related

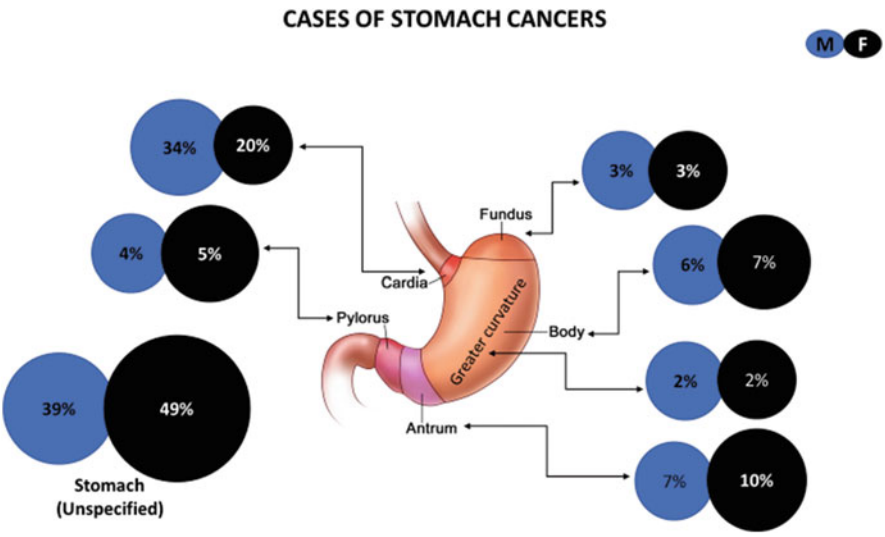

Fig. 7.1 Prevalence of stomach cancers in male and females

gastrointestinal cancers. The lack of early clinical signs of GI cancers leads to the development non-curable advanced disease state (Seidlitz et al. 2019) and as a result gastric cancer (Ferro et al. 2014) is now the fifth most common malignancy and the second leading cause of cancer-related deaths worldwide (Ferlay et al. 2010). According to The Cancer Genome Atlas (TCGA) Research Network, gastric adenocarcinoma can be divided into four different molecular subgroups (Fig. 7.1) (Ferro et al. 2014): those positive for the Epstein-Barr virus (EBV) with frequent *PIK3CA* mutations and *CDKN2A* silencing, a microsatellite instable (MSI) subtype with a hypermutation phenotype, a genomically stable (GS) subtype displaying diffuse histology and frequent *CDH1* and *RHOA* mutations and a chromosomal instable (CIN) subtype displaying aneuploidy and frequent mutation of *TP53* as well as activation of the receptor tyrosine kinase (RTK)-RAS pathway. Molecular characterization of AEG revealed their high similarity to the CIN subtype of gastric cancer (Ferlay et al. 2010) (Table 7.1).

In the last 20 years the incidence of gastric cancers dropped steadily but stomach cancer is still the second most prevalent cause for cancer related deaths worldwide (International Agency for Research on Cancer 1994). *Helicobacter pylori* (*H. pylori*) is considered as a class-I carcinogen and this represents a direct co-relation between infections with H. pylori and neoplastic transformations in the human stomach and one of the strongest known risk factors for gastric cancer (Correa 2013). *H. pylori* infections in humans induce both histological types gastric cancer (diffuse or intestinal types). Most important bacterial determinant that plays an important role in the development of gastric cancers are cytotoxin-associated gene pathogenicity island (*cag*PAI), vacuolating cytotoxin A (VacA), adhesion factors such as blood group antigen-binding adhesin (BabA) and sialic acid-binding adhesin (SabA)

Table 7.1 Data from cancer genome atlas

• Male prevalent • Located mainly in fundus and body • EBV-CIMP • Silenced CDKN2A • Amplified JAK2, CD274, PDCD1LG2 and ERBB2 • Enhanced immune cell signalling • Mutated Arid1a (55%) and BCOR (23%) mutated PIK3CA (80% subtype)	• Female prevalent • Gastric-CIMP • Silenced MLH1 • No such amplification • Mitotic pathway activation • Substitution mutations in TP53, KRAS, ARID1A, PIK3CA, ERBB3, PTEN AND insertion-deletion mutation in RNF43, B2M AND NF1 genes	• Diffusive in nature • Early age • Recurrent CDH1 (37%), mutated RHOA (15%) • Inactivated ARID1A • Integrin and syndecan mediated signalling are enhanced, increased angiogenesis	• Mainly found in gastro-esophageal junction/ cardia • Amplified RTK-RAS

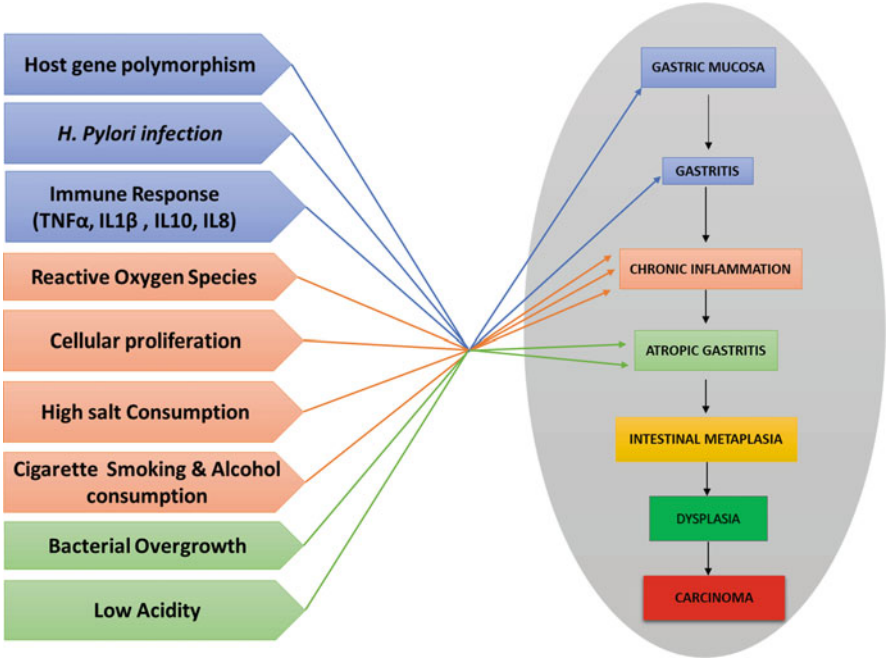

Fig. 7.2 Factors responsible for development of gastric cancer

(Boquet and Ricci 2012; Cover and Blanke 2005; Gerhard et al. 1999; Yamaoka et al. 2006; Sokolova et al. 2013) (Fig. 7.2). By activating one of the key regulators of inflammation such as nuclear factor kappa B (NF-κB), *H. pylori* activates cytokine signalling mediated by pro-inflammatory cytokines such as IL-8, TNF-α and STAT3, and drives the development of severe chronic inflammation and subsequently carcinogenesis and invasive carcinoma (Schweitzer et al. 2010; Aihara et al.

1997; Sharma et al. 1998; Beales et al. 1997; Suganuma et al. 2008; Ernst et al. 2008; Merchant 2008; Echizen et al. 2019). In addition, TNF-α promotes gastric tumor formation by activating a protein called NOXO1 (component of the NOX1 complex) which produces reactive oxygen species (ROS) and damages tissues. NOX1/ROS signalling induces gastric epithelial stem cells to multiply uncontrollably, resulting in tumor formation (Rhyu et al. 1994).

In addition, less than 10% of the cases of gastric cancer can be linked with inheritance. For example, familial inheritance of E-Cadherin is responsible for hereditary diffuse gastric cancers (HDGC). People with HDGC caused by CDH1 gene mutations are born with abnormally short, non-functional version of E-cadherin or alter the protein's structure. Specific genes such as MCC, APC, and p53 tumor suppressor genes are also involved in increasing the risk of gastric cancer (Trédaniel et al. 1997). Gastric adenocarcinoma, proximal polyposis of the stomach (GAPPS), and familial intestinal gastric cancer (FIGC) are other major syndromes accounting up to 3–5% of hereditary familial gastric cancers. There are also environmental and behavioral factors that could increase the risk of gastric cancers. Smoking, a high level of salt and processed meat in diet and high consumption of alcohol has been shown to increase the chances of gastric cancer development (Graham et al. 1990; Risch et al. 1985; Buiatti et al. 1989, 1990; van den Brandt et al. 2003).

As mentioned in the beginning of the chapter probiotic microorganisms show anti-cancer activities and they attribute these anti-cancer activities to mainly maintaining host-microbial balance of the intestine, reducing pathogenic and carcinogen producing microorganisms and their enzyme activities, clearing carcinogens by binding on them. Furthermore, several studies have shown the potential of probiotics in immune modulation, enhanced gut barrier function, anti-inflammatory effects, and reducing tumor formation and metastasis (Servin 2004; Cotter et al. 2005). Although more research is required for determining functional efficacy of probiotics on cancer prevention and treatment, probiotics can be still be considered as a potential nutraceutical and in this chapter, we will explore some of the established effects of probiotics on GI cancers.

7.2 Types of GI Cancer, Associated Risk Factors, Diagnosis and Treatment

7.2.1 Pancreatic Cancer

Pancreas is a glandular organ of body comprised of endocrine and exocrine components. The endocrine system secretes out insulin, glucagon, and somatostatin while exocrine part secretes different enzymes for digestion of food. Pancreatic cancer shows three phases: acute, chronic, and metastasis (aggressive, chemo-resistant) pancreatitis (Singhal et al. 2016).

7.2.2 Liver Cancer

The function of a liver in body is to aid in digestion process. A healthy liver could start to develop liver damage or uncontrolled cell growth that could lead to two stages of liver cancer (a) benign tumor and (b) hepatocellular carcinoma (HCC)—malignant stage. HCC is frequent in patients with liver cirrhosis. The major risk factors for the development HCC are chronic viral infections mediated by either hepatitis B or hepatitis C or excessive alcohol consumption (Aham et al. 2013; Batey et al. 1992; Morgan et al. 2004). The diagnosis of liver cancer starts with the identification of liver cirrhosis in patients that gives a possibility of presence of tumor. An ultrasound test is done to identify the nodule as well as blood test is done to screen out a protein called α-fetoprotein. If the higher level of this protein is present in blood, it gives a clue of liver cancer (Johnson 2001). The symptoms encircle weight loss, fatigue, nausea/vomiting, fever, swelling of abdomen, jaundice, enlarged liver, etc. The diagnosis includes blood tests, radiological and histopathological examination.

7.2.3 Stomach Cancer

The tissue lining (mucosa) of the stomach is prone to cancer because it remains the site of initiation of cancerous tumor cells. Various types of stomach cancers such as gastric lymphomas originate from cells of immune system present in stomach wall. They are usually non-Hodgkin lymphomas. Gastrointestinal stomach tumors (GIST) are rarely occurring tumors which originate from the tissue of inner lining of stomach and intestine. Neuroendocrine tumors are believed to originate from nervous or endocrine cells of stomach. Environmental factors, nutritional factors and *H. pylori* infection have been implicated in the development of the stomach cancer (Sasazuki et al. 2006). Diagnosis of stomach cancer is not very predictable as symptoms are subtle and simple like abdominal pain, indigestion, vomiting, weight loss, blood in stool, poor appetite, or swelling of abdomen. Clinical examination including detection of tumor markers such as carcinoembryonic antigen (CEA), the carbohydrate antigens (CA)—CA19–9, CA-72-4, CA125, CA24–2 and alpha fetoprotein, endoscopy, endoscopic ultrasound, radiological examination as well as histopathological examination remains the choice of diagnosis of stomach cancer (Tong et al. 2016; Kotzev et al. 2018). The treatment plan includes surgery with removal of stomach or total gastrectomy, removal of lymph nodes or/and removal of other related organs (Otaka et al. 2006). Adjuvant therapy (a combination of chemo and radiotherapy) is given in addition to surgery (Ushijima et al. 2004; Earle et al. 2002).

7.2.4 Colorectal Cancer

Unregulated growth of cells in colon or rectum is called colorectal cancer (CRC). The colon and rectum together (colorectum) constitute the large intestine and the final parts of the GI tract. CRC is sometimes referred to as bowel cancer. The former part of large intestine is colon which is divided into four sections: (1) ascending colon: starts from cecum and extends to right side of the abdomen. (2) transverse colon crosses the body from right to left side and is known as proximal colon (3) descending colon extends to left side (4) sigmoid colon named after its shape, i.e. "S" which is the final portion of the colon and then joins rectum.

Early CRC often has no symptoms and as the tumor grows, it may obstruct the intestine resulting in blood loss. Bleeding from the rectum, blood in the stool, dark or black stools, change in bowel habits, weakness, excessive fatigue are the additional warning signs. CRC starts with noncancerous cellular growth termed as polyp which grows attached to the inner lining of the colon or rectum. The most prevalent type is adenomatous polyp or adenoma (Amersi et al. 2005) which arises from glandular cells whose function is to lubricate the colorectum. Although all adenomas have potential to grow as cancerous cell but only 10% are invasive cancerous cells. This type of tumorous growth is termed as adenocarcinoma. These can proliferate and invade through blood vessels or lymph vessels and via this means they spread fast.

7.2.4.1 CRC Stages Using the Surveillance, Epidemiology, and End Results (SEER) Summary Staging System

In Situ In this stage cancers have not yet begun to invade the wall of the colon or rectum.

Local Cancers that have grown into the wall of the colon or rectum, but have not extended through the wall to invade nearby tissues.

Regional Cancers that have spread through the wall of the colon or rectum and have invaded nearby tissue, or that have spread to nearby lymph nodes.

Distant Cancers that have spread to other parts of the body, such as the liver or lung.

The risks (Amersi et al. 2005) associated with the development of colorectal cancer are enlisted here.

Age Age is one of the prime factors that shows an increasing age has higher risk of acquiring CRC but affects men and women in different age groups. Men tends to acquire the disease at the age of 68 whilst women in 72 and later. The median age of CRC diagnosis is about 40–50 years (Kemppainen et al. 1993; MacGillivray et al. 1991).

Gender CRC is more prevalent in men than in women where they are reported with 30% higher risk of the disease. There could be unidentified reasons for the gender disparity in disease occurrence (Wei et al. 2004).

Ethnicity or Race Worldwide non-Hispanic blacks are more prone to the disease while this occurrence was lowest in Asians and Pacific islanders. Alaska natives specifically rural natives are at higher risk of infection by *H. pylori* and hence associated risk of cancer in colon or stomach (Parker et al. 1998; Freeman et al. 2002).

Family History of Colorectal Cancer There is a risk of 30% a person acquires the disease from the family line with the case history of CRC. The risk is higher for the people with many first-degree relatives suffering from the same (Lynch et al. 2003).

Personal Medical History: Chronic Inflammatory Bowel Disease When colon remained inflamed over a long period of time it results in chronic inflammatory bowel disease. Most common forms of the diseases are ulcerative colitis and Crohn's disease. There are increased chances of developing the cancer if the inflammation persists (Bernstein et al. 2001; Gyde et al. 1988).

Personal Medical History—Diabetes The persons with type 2 diabetes are at higher risk of attaining the CRC. Dysregulation of insulin signalling and glucose control, leading to hyperinsulinemia and inflammation, are important biological pathways for the development of this cancer (Peeters et al. 2015; Mills et al. 2013).

Obesity Obese people are at prior risk of getting CRC. Lower body and metabolic activities may lead to the deposition of fats. Although obesity is associated with an increased risk of colorectal cancer, individuals with obesity without raised insulin levels do not have elevated risk of CRC (Bardouet al. 2013; Ma et al. 2013). However, lean individuals with hyperinsulinemia had an increased risk of colorectal cancer of a similar magnitude to individuals with obesity and hyperinsulinemia.

Diet Few dietary elements are directly linked to regulate CRC like calcium intake and dairy products decline the risk of adenomas and CRC, fibers prevent colon cancer, folate intake with diet promptly inhibits the risk of new tumor formation, fruits and vegetables contain dietary fibers and nutrient that decreases the risk of tumor formation (Terry et al. 2001; Baron et al. 1999). But intake of red and processed meat influence to greater risk of colorectal cancer which is related to some ingredients in the meat. Vitamin D could downregulate the occurrence of CRC (Kampman et al. 1999; Fuchs et al. 1999).

Consumption of Alcoholic Beverage and Smoking Heavy usage of alcohol and smoking leads to development of CRC (Verma 2009; Cho et al. 2004).

7.3 Role of *H. pylori* in Gastrointestinal Cancer

It is said that *H. pylori* originate in Africa and over 50% population worldwide is affected from *H. pylori* but it is more prevalent in developing countries. The bacterium can be acquired right from the childhood showing symptoms of gastroenteritis. The *H. pylori* infection also leads to deficiency of vitamins like A, B12, C, E and micronutrients such as copper and iron. A lot of studies have revealed how *H. pylori* is infectious to human stomach and digestive tract. Among various mechanisms one is alteration of antioxidant properties of melatonin because they scavenge the available antioxidants in the host body but they are reduced in number if higher concentration of ascorbic acid is available in the host. Ultimately, the bacterium is able to change the redox potential of these antioxidants and leading to oxidative stress in the digestive system. Several diseases could develop along with the *H. pylori* infection in the human, viz. (1) higher chances to acquire cholera disease, (2) development of acne and induce infection by Acne vulgaris (etiological agent of acne), (3) HP infection onset the hyperprolactinemia which later induces Polycystic Ovarian Syndrome (PCOS), (4) rise in blood pressure level, (5) escalated risk of ischemic heart disorder, (6) higher risk of attaining diabetes, (7) post treatment weight gain. *H. pylori* is a potential carcinogen (defined by WHO) due to certain reasons, viz. (1) it leads to the development of adenocarcinoma, and (2) MALT (mucosa associated lymphoid tissue) lymphoma (Alfarouk et al. 2019). In addition, the development of gastric cancer is also dependent on health and immune status of the host as well as the nature of the *H. pylori*.

The onset of *H. pylori* mediated carcinogenesis includes several genes and related factors in the microenvironment. Following is the listed factors that are involved in the GI carcinogenesis related to *H. pylori*:

Ureases enzyme (cytoplasmic enzyme) creates a suitable microenvironment for the *H. pylori* bacteria to grow and establish in the acidic environment of stomach because the enzyme catalyzes the substrate urea and releases ammonia and carbon dioxide which neutralizes the acidity and buffers the acid neutralized environment that becomes suitable for the growth of bacteria. Facilitated diffusion is mediated by urease enzyme in the mucus membrane. Ureases also alter the host immune response towards *H. pylori* but not the pathogenesis of the bacteria. A transporter has been identified encoded by the *ureI* gene capable of delivering urea to the cytoplasm where urease enables neutralization and buffering capacities. Hence, it is invincible that urease enzyme is an important factor that facilitates the establishment of *H. pylori* (Weeks et al. 2000). Acidic pH prevails bacterial enzyme synthesis such as arginase and carbonic anhydrase (MacGee et al. 1999, 2008).

Arginase is involved in providing the substrate to urease to produce L-ornithine and urea.

Carbonic anhydrase (zinc containing metalloenzyme) catalyzes the interchangeable reaction of carbon dioxide and water to form carbonic acid; this gets dissociated to form bicarbonate liberating hydrogen ion (H^+). The enzyme is omnipresent in both prokaryotes and eukaryotes in cytoplasm and organelle like mitochondria and cytoplasmic membranes. The CA enzyme is functionally related to buffering and

biosynthetic processes in cells. Two different forms of CA exist in *H. pylori* (a) α-type CA (HpaCA) and (b) HP β-CA (HpbCA). HpaCA encourages urease activity and HpbCA is associated with growth of bacteria in acidic environment.

H. pylori expressed Lewis antigen is a constituent of lipopolysaccharide of bacterial (*H. pylori*) cell wall. The Lewis antigen is a part of human blood group that is encoded by the genes present in chromosome 19 p13.3 (FUT3 or Lewis gene). *H. pylori* expressed Lewis antigen mimics the human type Lewis antigen because the O-antigen (side chain of LPS) shows homologous structure with that of the human Lewis antigen. The mimicry allows the *H. pylori* to overcome the immune defense system of the human beings. This also facilitates the adherence of *H. pylori* to the gastric epithelial mucosal cells. But humans that have blood group A and B are likely to resistant to infection by the *H. pylori*. The expression of Lewis antigen in different *H. pylori* strains is different with genes such as Lex, Ley, both, Lea, Sialyl-Lex or negative for both. Lex and Ley are correlated with cagA+ and s1/m1 VacA. Among the western population, the dominant phenotypes are LeX and Ley while Lea and Leb are found in a smaller proportion. Possessing of Lex and Ley leads to higher *H. pylori* internalization rates by gastric epithelium as compared to Lea and Leb or non-expressing Lewis antigen.

VacA is a vacuolating cytotoxin encoded by vacA gene that is secreted out. All strains of *H. pylori* contain vacA gene but all may show variable expression. Secretion of VacA is prominent in patients suffering from GI cancer. The toxin is made of two subunits say, P55 and P33 proteins. The former is responsible for creating pores in the epithelial layer while the latter one disintegrates mitochondrial fission system. These lead to cellular death of the epithelial tissue. Maturation and sorting of lysosomal enzymes are adversely affected by the VacA which also inhibits T-cells population.

Cag A (Type IV protein secretion system (*T4SS*) encoded by cagA gene) delivers cagA-oncoproteins which decline apoptosis. The usual target of cagA is mitochondria that lead to respiratory impairment of cancer cells. The HP induced pathogenesis is heterogenous in the way that if cagA+ is in association with the gastric adenocarcinoma development then either cagA+ or cagA- could induce B-lymphoma which triggers IL2 expression via T cells. cagA is also related to higher cytokines production rate. The cagA+ gene product alters epithelial activity because it now acts as phosphatase enzyme for catalyzing dephosphorylation reaction that induces a pro inflammation with release of IL-8, MAPK, and NF-BK which are signature of carcinogenicity.

Exterior proteins (*BabA2*) is a membrane exterior protein whose presence is an indication of GI cancer and is encoded by babA2 gene. It shows binding with Lewisb (Le b). The presence of combined proteins, i.e. BabA2, CagA, and Vac A indicates the probability of carcinogenesis.

7.4 Key Elements of Humans in Response to *H. pylori* Induced Gastric Cancer

Human body shows response to inflammation caused by *H. pylori* in several modes showing a variety of elements that are produced at the onset of the cancer. Few are enlisted:

β-catenin: It is a CTNNB1 gene encoded protein present on band p12 at short arm of chromosome 3. This is the region which gets affected by somatic alterations in tumors. This protein coordinates for cell–cell adhesion as well as gene transcription. β-catenin is a proto-oncogene that has been found to accumulate inside the nucleus in precancerous lesions of gastric cancer. β-catenin is related to vivid kinds of tumors, viz. hepatocellular carcinoma, ovarian carcinoma, breast cancer, lung cancer, colorectal cancer, basal cell carcinoma, prostate cancer, pilomatrixoma, medulloblastoma, head and neck squamous cell carcinoma, and glioblastoma.

Epidermal growth factor receptors (*EGFR*) are proteins in relation to tyrosine kinase receptor like Her 1, Her 2, Her 3, and Her 4. These are cell surface receptor proteins that are expressed in many of the carcinomas.

Immunological response towards H. pylori: mutagenic substances are formed in response to tumorigenic inflammation by *H. pylori* such as nitric oxide synthase enzyme (iNOS) which releases free reactive nitrogen species that diminish antioxidative agents.

Enzyme phospholipase A_2 (PLA_2): this enzyme catalyzes the reaction involving fatty acids releasing off arachidonic acids that later convert into prostaglandins and leukotriene with the help of enzymes cyclooxygenases and lipoxygenases. Prostanoids are produced from prostaglandins by the action of cyclooxygenase 1 and 2. The role of prostanoid is to increase the production of pro-aggregatory prostanoid, thromboxane via platelets which all induce TNF-α, TNF-γ and IL-1 that are related with colorectal cancer. Leukotriene is related to gastritis whose receptors are expressed in gastric cancer cells.

7.5 How Probiotics Interrupt Gastrointestinal Cancers

Probiotics have been used to manage a number of GI disorders such as diarrhea, infection, and inflammation. There are several ways that probiotics defend our body from cancer (Fig. 7.3). Among all the mechanisms available in literature one is replacement of gastrointestinal microflora, deactivation of carcinogens, competitive interaction with pathogenic microorganisms, enhancement of immunity, antiproliferative action on carcinogens such as apoptosis and tissue differentiation, aids food digestion, inactivation of tyrosine kinase signal pathways (Li et al. 2014; Chen et al. 2012; Del Giudice et al. 2009; Yang et al. 2012; Hsieh et al. 2012). These and other mechanisms by which probiotics could inhibit the chances and/or onset and progression of GI cancers are given in Table 7.2.

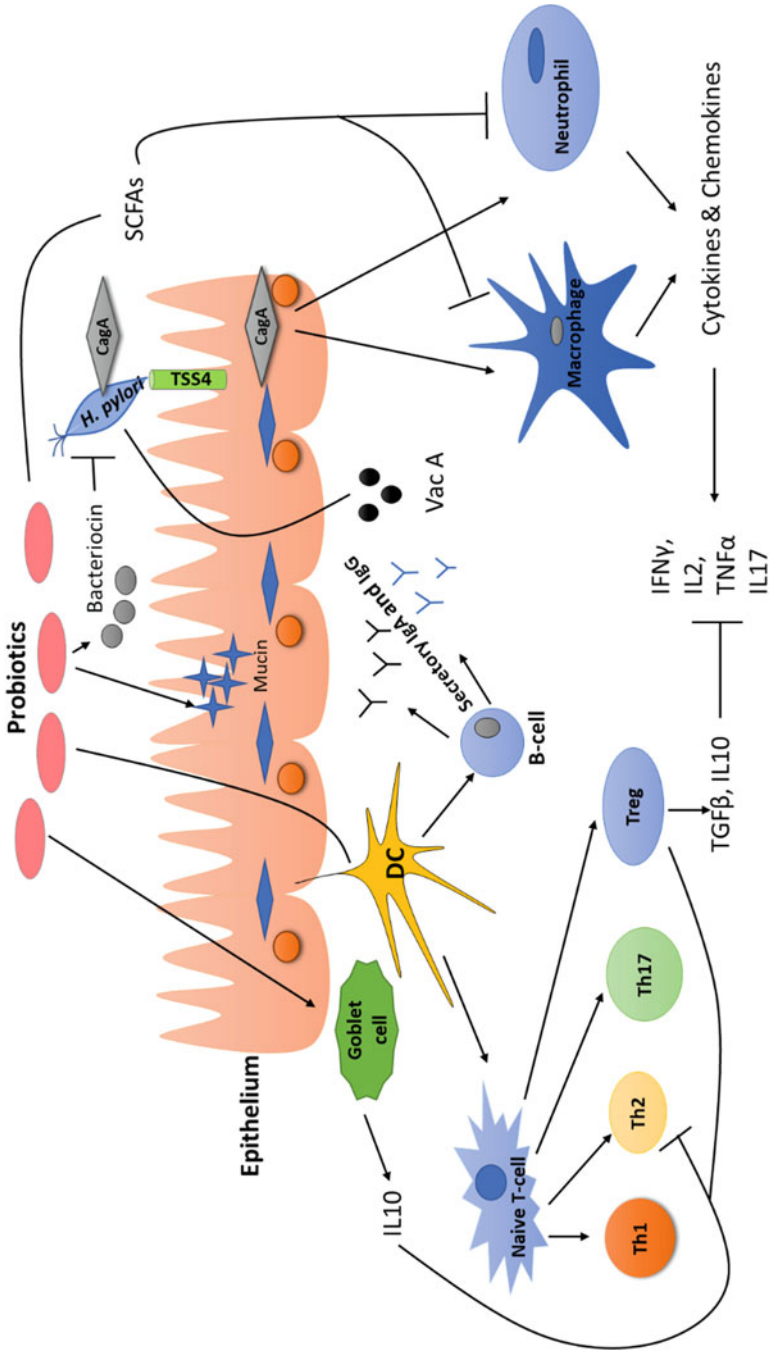

Fig. 7.3 Possible mechanism of actions of probiotic bacteria in the immunomodulation and the anti-inflammatory response in the gut. Macrophages, dendritic cells and other immune cells may involve in preventing pathogens, gastric inflammation and cancer

Table 7.2 Combating risk factors of GI cancer with aid of probiotics

Risk factors prone to GI cancer	What probiotics play	References
Gut microbiota (increase in opportunistic pathogens) *bacteroides, fusobacterium, salmonella, prevotella*	Replacement with LAB group, *Bifidobacterium, Lactobacillus* which even declines the population of *Escherichia* and *Staphylococcus*	Marteau et al. (2001)
Disturbed physio-chemical conditions in GI organs viz. incomplete fermentation or digestion, alkalosis, water absorption rate, toxic fecal water content	Probiotics facilitates fermentation and decreases pH while simultaneously decreases products like putrescine, cadaverine and tryptamine (substances shows putrefaction)	Lebeer et al. (2008)
Epithelial line damage (show higher permeability along with pathogen translocation, rearrangement of proteins at tight junction)	Recovery of damage in epithelial barrier by specialized proteins like defensins, heat shock proteins (cryoprotective) along with mucus production that leads to normal survival of epithelial cells	McBain et al. (2001)
Increased production of bacterial enzymes that harm the gut (β-glucoronidase, β-glucosidase, azo-reductase, nitro-reductase, alcohol dehydrogenase)	Population of bacteria producing the harmful enzymes is reduced (*Clostridium, Bacteroides, Salmonella, Citrobacter, Escherichia, Enterobacter, Enterococcus, Escherichia, Staphylococcus, Streptococcus*)	Weisburger (1971)
Production of carcinogenic metabolites (iq, tryptophanase, urease, acetaldehyde, mnng, n-nitroso compounds, sodium azide, aromatic amines, aflb1, trp-1, transformed bile salts, indoles, aglycones hydrogen sulfide, benzo–α-pyrene)	Destabilization of carcinogens: production of antioxidants and enzymes that detoxify the carcinogenic metabolites either by binding to it or deactivating them (GTS, catalase, glutathione, glutathione reductase, superoxide dismutase, glutathione peroxidase)	Waldecker et al. (2008)
Dna damage activity is increased (absurd cell growth, tumor development, dysplasia)	Production of anticancerous metabolites by probiotics (phenols, CLAs, SCFAs) shows increased apoptosis and differentiation in cancer cells	Azad et al. (2018)
Development of inflammation in intestine (higher production of nf-κb, il-8 and il6), this leads to decreased immune response towards tumor cells	Lowers intestinal inflammation by lowering TLR-4 and activating immune response against those tumor cells, activation of regulatory T cell, increased bactericidal phagocytosis stimulation of DCs and natural killer cells	Zhong et al. (2014)

7.5.1 Mechanisms of Probiotics Altering the Onset of GI Cancer

The metabolic process in the stomach and small intestine get shifted with the changes of micoflora present thereby the replaced microflora also replaces the metabolites, pathways and enzymes in the system. In liver, metabolism of

glucuronide conjugation occurs where conjugation of glucuronic acid takes place resulting in liberation of polar metabolites. The process of deconjugation of these polar metabolites takes place in intestine through bacterial enzymes (β-glucuronidase) releasing aglycones which is a potent carcinogen. Few other examples of these enzymes are azo-reductase and nitro-reductase which also release toxic carcinogenic metabolites in the intestine (Marteau et al. 2001). The consumption of probiotics or synbiotics reduces the potential risk of cancer development by replacing these enzymes which ultimately results in different metabolic pathway. Probiotic food like yogurt contains *lactobacilli* that replace the fecal enzyme if taken simultaneously for few days (Uccello et al. 2012). In a research conducted by Marteau et al. (2001) a decline in nitro-reductase activity was observed after taking dairy products including *L. acidophilus*, *B. bifidum,* and mesophilic cultures (*Streptococcus lactis* and *Streptococcus cremoris*) but the two enzyme β-glucuronidase and azo-reductase did not alter.

7.5.1.1 Carcinogens Replacement by the Probiotics

Consumption of red meat is one of the risk factors associated with CRC because when it is cooked at much elevated temperature heterocyclic aromatic amines (HCA) are produced which later involves the intestinal microbiota to further liberate mutagenic metabolites such as 3-amino-1,4-dimethyl- 5H-pyrido-[4,3-b] indole [Trp-P-1], 3-amino-1-methyl-5Hpyrido-[4,3-b]indole [Trp-P-2], 2-amino-3-methylimidazo [4,5-f] quinoline [IQ], 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine [PhIP], 2-amino-3,4-dimethylimidazo[4,5-f] quinoline [MeIQ], and 2-amino-3,8-dimethylimidazo[4,5- f]quinoxaline [MeIQx] (Rhee et al. 2001). The release of such mutagenic compounds interacts with mucosa of colon and leads to tumor formation. When probiotic microorganisms are present in the intestine, they bind to these mutagenic compounds and modify them so these could not have an impact on the colonic mucosa. In a study, the genotoxicity was downregulated by oral administration of probiotic strains of *L. acidophilus* and *B.* spp. which was found to bind with the mutagen Trp-P-1 irreversibly (Rafter et al. 2007).

7.5.1.2 Competitiveness with the Pathogenic Microflora

The colon region of the GI tract is the most burdened with bacteria. These bacteria are usually normal flora but could be an opportunistic pathogen which could lead to initiation of acute or chronic disorders. The diet of a person reflects the type of microflora harboring in the gut; as already mentioned, red meat intake leads to production of carcinogens but also alters microbiota which increases the population of sulfate reducing bacteria that produces hydrogen sulfide (genotoxic compound). Some putrefactive bacteria also reside in the colon that creates an environment for onset of CRC namely, *Clostridium* and *Bacteroides* spp. A researcher found a decline in the fecal flora of polyp and colon cancer patients after they were administered with certain group of LAB (*Bifidobacterium*, *Lactobacillus* sp.), simultaneously there was a decline in the number of Clostridium sp. (Gianotti et al. 2010).

7.5.1.3 Probiotics to Improve Host Immune System

The suppression of tumor and its progression is largely undertaken by host immune system. An array of immune system components plays their role such as antigen presenting cell (APCs), natural killer cells, T and B cells but apart from these probiotics are believed to improve the immunity of a person and is also evident from several research done in this regard. A strain designated as *L. casei* Shirota (LcS) was observed among other potential LAB that suppressed transplantable mouse sarcoma (Yokokura et al. 1981). This strain does not directly suppress tumor but enhance the immunity of the host by increasing the number of cytokines, viz. Interferon- γ (INF-γ), Interleukin –β (IL-β), and tumor necrosis factor-α (TNF-α), thus tumor is suppressed (Fig. 7.3). The suggested mechanism behind the immunity improvement is when probiotics are ingested by a person it binds to M cells in Peyer's patches, macrophage or dendritic cells (DCs). Then the phagocytic process is triggered to digest these LcS several cytokines are generated. Thereafter, the LcS digested components are recognized in Peyer's patches by toll like receptor-2 in APCs wherein again several cytokines are generated. Side by side natural killer cells do play a vital role in activating immunity. Natural killer cells are granular lymphocytes which are bone marrow derived cells and are activated by probiotics. In a double-blind study, a mixture of two probiotic species *B. longum* (*BB536*) and *L. johnsonii* (*La1*) was administered to the patients and it was found that *La1* reduces the concentration of gut pathogens and modulates the local immunity by adhering to the colonic mucosa (Culligan et al. 2009).

7.5.1.4 Effects on Apoptosis and Tissue Differentiation

Apoptosis is defined as a genetically programmed process of regulating cell numbers that control vital role in preventing cancer. In many cancer cases, apoptosis does not initiate in response to uncontrolled cell growth. In this regard, probiotics provide a great help in controlling proliferative growth of cancer cells or initiating apoptosis.

7.5.2 Variety of Probiotic Microorganisms Showcase Anticancerous Action Towards GI or Colon Cancer

Antitumor activity is studied on animals using a variety of probiotic bacteria; *B. longum* to show inhibition of liver cancer in mice, simultaneously *B. infantis* and *B. adolescentis* when injected subcutaneously and intravenously into those mice had shown antitumor action (Wei et al. 2018). In a randomized controlled trial, the effective role of probiotics against acute gastroenteritis was done in selected group of infants and adults to judge the declination in HP via probiotics using array of probiotic bacteria, namely, *L. rhamnosus* strain GG, *Enterococcus faecium* SF68, *Saccharomyces boulardii, L. reuteri*, and yogurt (a traditional probiotic food) (Marteau et al. 2001). Probiotic microorganisms such as *L. acidophilus, L. gasseri, L. confuses, Streptococcus thermophiles, B. breve* and *B. longum* reduce genotoxicity in GI cancers after administered orally mainly via inhibitory actions against N′-nitro-N-nitrosoguanidine (MNNG) and 1,2-dimethylhydrazine (Consoli

et al. 2016). Probiotics are capable of modulating the gut microbiome along with systemic and immune response of the consumer (Table 7.3). Probiotics not only have the capability of preventing and inhibiting the carcinogenic agents but they are equally effective in preventing complications of cancer treatments. Probiotics induce these effects partly by producing soluble compounds that may interact directly with tumor cells in culture and inhibit their growth (Wan et al. 2014).

7.5.2.1 Probiotics in Colon Cancer

The colon region of GI tract harbors a vast number of bacteria whose composition may affect the chances of cancer. The replacement of these microflora by probiotics prevents the onset of carcinogenic activities. Induction of cell apoptosis by *L. rhamnosus* in animal model with colon cancer is reported. Inside colon the fermentation of prebiotics releases short chain fatty acids (SCFA) like butyrate which is significant in ulcerative colitis. In addition to this, sodium butyrate is a strong inhibitor of growth and inducer of phenotype differentiation and apoptosis while reduces risk factors involving colon cancer and adenoma. In a clinical trial conducted in 2016 further demonstrated that randomized oral administration of the probiotic *Saccharomyces boulardii* in CRC patients downregulates pro-inflammatory cytokines (Yu et al. 2016).

7.5.2.2 Breast Cancer

It has been reported that the routine consumption of probiotic *Lactobacillus casei* enhances the immune response of breast cancer patients. Nanotechnological interventions with probiotics (*L. plantarum*; yogurt) have shown improved production rate of cytokines IFN-γ, TNF-α and IL-2 and NK cells (Mendoza 2019).

7.5.2.3 Bladder Cancer

It has been shown in a research that intake of probiotics is efficient against bladder cancer. Transurethral Resection of Bladder Tumor (TURBT) is a procedure in which bladder tumors can be removed but recurrence of the tumors can be observed regularly. After TURBT, as compared to the intravesical epirubicin alone, oral administration of *L. casei* with combination of intravesical epirubicin reduces recurrence rate of bladder tumors more significantly (Sharifi et al. 2017; Shah 2007; Naito et al. 2008; Aso et al. 1992; Panebianco et al. 2018).

7.5.2.4 Other Cancers

Recently, few other studies have suggested the beneficial effects of probiotics on other GI cancers. Probiotics could prevent pancreatic cancer by modulating pancreatitis, diabetes, pancreatic necrosis, inflammation, and obesity (Rafter 2003; Gamallat et al. 2016; Olah et al. 2002, 2007; Liong 2008; Kim et al. 2008). In vivo studies with mice have been reported that probiotic administration could inhibit progression of hepatocellular carcinoma (HCC). Even liver-tumor size has been reduced when tumor injected mice were supplemented with probiotic mixture.

Table 7.3 Probiotics to encounter cancer and other related infections

Role played	Probiotics	Mechanism	Reference
Antioxidant activity	*B. longum, L. acidophilus*	Inactivate ROS via enzymatic mechanism (coupled NADH oxidase, peroxidase and catalase)	Azad et al. (2018)
Immune response improvement	LAB: *L. acidophilus*	Release of cytokines, IL-10, down regulation of IL-2 and TN-α, activation of CD4+ T cells.	Azad et al. (2018)
Short chain fatty acid production	*Buty vibrio fibrisolvens* MDT-1, *Propionibacterium acidipropinici* (propionate and acetate producer)	Produces high amount of butyrate which reduces aberrant crypt foci (ACF) in mouse model of colon cancer, reduces glucuronidase activity	Zhong et al. (2014), Wei et al. (2018)
Anticarcinogenic, antimutagenic and antioxidative effect	Kefir: fermented kefir grains with *L. paracasei, L. kefiri, L. parabuchneri, Acetobacter lovaniensis*; yeast: *Saccharomyces cerevisiae* and *Kluvveromyces lactis*	Bacteria convert lactose to lactic acid thus decreasing the milk pH, yeasts produce ethanol and CO_2, kefir is also rich source of vitamins (A, B1, B2, B5, C, B12 and folic acid) and amino acids (serine, lysine, alanine, threonine, tryptophan, valine, lysine, methionine, phenylalanine and isoleucine). Bioactive peptides in kefir induce activation of macrophages and phagocytosis and nitric oxide production along with production of TNF-α and cytokines (IL-5, IL-6, IL-1α, IL12)	Consoli et al. (2016)
HP infection suppression, antidiarrheal, anticarcinogenic, improves lactose metabolism	Traditional yogurt: a probiotic dairy product made out of fermentation of milk using starter cultures of *L. acidophilus*, *Bifidobacterium* sp., *L. casei*; now other bacteria used are *L. casei* Shirota, *L. rhamnosus* GG, *L. reuteri* along with the aforesaid	Lactic acid produced by these bacteria shows antimicrobial activity, reduced colonization of HP, decreases the level of certain enzymes such as β-glucuronidase, azo-reductase and nitro-reductase. Produces IF –α and NK cells	Wan et al. (2014)
RS retards tumor growth in pancreatic cancer	Prebiotics: resistant starch	Resistant starch promotes the growth of bacteria involved in butyrate production	Yu et al. (2016)

7.6 Conclusion

Recent studies have supported the idea that probiotic consumption may involve in immunomodulation, reduction of tumor development and establishment of healthy gut in gastric cancer patients. Probiotic microorganisms in formulation have the anti-toxic and anticarcinogenic potential that eliminates toxics and tumorigenic substances produced after digestion in the gut. Probiotics bacteria have the ability to modulate the immune system by alteration of the cytokines production and signalling pathways related to epithelial cell inflammation and tumor initiation. Although there is still lack of direct evidence of how probiotics induces its actions, however research in this field still has to progress towards a concrete understanding of molecular mechanism of the microorganisms with human hosts. Current therapies that are available for different types of GI cancers includes chemotherapy, radiotherapy immunotherapy and targeted therapy comes with diverse side effects in patients.

Now we are looking for safe natural products as an alternative to the conventional drug-based therapy for routine health care and disease management. Probiotics have recently emerged as safe, cost-effective and easily affordable as prophylactics for management of gut related inflammatory disorders. Therefore, probiotics would be used as biotherapeutic agent for the treatment of cancer and other inflammatory diseases which can maintain a homeostatic balance between inflammation and tumor progression.

Furthermore, the use of modern biotechnology approaches to construct designer probiotics to achieve targeted health benefits is vital in the present era. The designer probiotics may have promising results against gastric inflammations and other related disorder due to their unique ability to modulate and regulate the host's immune response by initiating the activation of specific genes in and outside the host intestinal tract. The aim of this book chapter is to emphasize the promising beneficial impact of probiotics on human health for better lifestyle.

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An Update on the Probiotic Usage in Bacterial Vaginosis

8

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Abstract

Bacterial vaginosis is an inflammatory infection caused by the overgrowth or imbalance of bacteria that are naturally found in vagina. These types of infections are also known to increase the risk of contracting sexually transmitted diseases and pregnancy complications. The beneficial microflora predominantly consisting of *lactobacilli* plays a prime role in preserving and retaining the physiological state of vagina. The bacterial communication of normal microflora is altered by that of pathogenic bacteria in bacterial vaginosis which enables the expansion of pathogenic biofilms instead. This has been posing many challenges in terms of treatment and antibiotic resistance which also leads to recurrence of infection. This has motivated microbiologists to look for bio therapeutic alternatives and probiotics have been gaining a lot of attention in this aspect. Probiotics may confer health benefits during vaginosis and ameliorate physiological condition. In this book chapter, we look at the pros and cons of this alternative and the recent advances in this field.

Keywords

Antibiotic resistance · Bacterial vaginosis · Biotherapeutic · Inflammation · Infections · Lactobacillus · Pregnancy · Probiotics

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Abbreviations

BV	Bacterial vaginosis
FISH	Fluorescence in situ hybridization
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HBD 2	Human beta defensin-2
IFN γ	Interferon gamma
IL	Interleukin
IP 10	IFN γ- inducible protein 10
MIP-3α	Macrophage inflammatory protein-3 alpha
NFκB	Nuclear factor kappa-light chain enhancer of activated B cells
PCR	Polymerase chain reaction
PID	Pelvic inflammatory disease
SLPI	Secretory leukocyte protease inhibitor
STI	Sexually transmitted infection
TNF α	Tumour necrosis factor alpha

8.1 Introduction

Human microbiota is a huge integrated part of our health, as they co-exist in us within different axes and niches. Modern studies on population dynamics have taken a turn in the philosophy and debate of the *niche and neutral theories* that extends to human microbiota as well. Nevertheless, microbial homeostasis and symbiosis with human body is a major contributor to our health. The Human Microbiome Project has also revealed that our genetic material is largely occupied by the microbiota existing in our body by a 10:1 ratio (Barrientos-Durán et al. 2020). Hence, the symbiotic relation of the microbiota within our body is essential, as they are a part and parcel of our biological processes like metabolism. Many of their metabolites not only help us metabolize but also provide defence to fight other pathogenic microbes. This is done by keeping the microbial homeostasis in check, especially in our gut axis.

Neutral theory explains that species variation and evolution is due to random genetic drift and community is assembled in a neutral fashion.

Niche theory on the other hand, explains that similar species form unique assemblies through evolution, depending on their genes and environmental factors.

Bacterial vaginosis (BV) is a very common vaginal infection in women, mainly caused by an imbalance in the vaginal microbiota and is often associated with other health risks and complications. These include pregnancy complications and high

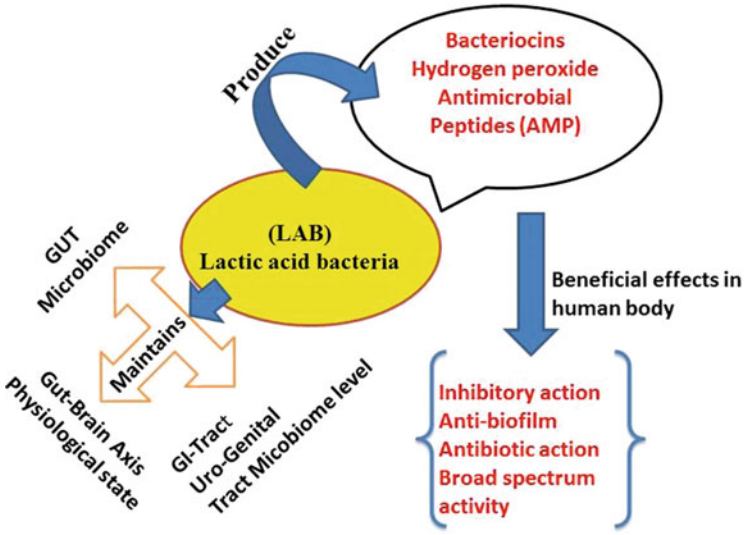

Fig. 8.1 Role of lactic acid bacteria in human body

risks of acquiring other infections like pelvic inflammatory disease (PID) and sexually transmitted infections (STI) (Ya et al. 2010). Studies trying to establish a link between BV and STI have always been inconclusive, although most of them have suggested the disturbance of microbial homeostasis and the pre-existing infection can easily attract other pathogens including the bacteria and viruses causing STI. Antibiotics like Metronidazole and Clindamycin are commonly administered either orally or as vaginal applications, but often come with side-effects like gastrointestinal disturbances, metallic taste in mouth, paresthesia, leucopenia, sensitivity reactions, etc. Apart from these side-effects, most of the treated cases go through antibiotic resistance and recurrence. Moreover, some bacterial biofilms persisting in the host body make the antibiotics nugatory. In pregnant patients, these antibiotic treatments mostly fail to reduce pregnancy associated complications. Other factors like smoking sexual activity, socio-economic status, and demographic distribution are also said to be the contributors of BV prevalence, although the reports are contrasting (Nelson et al. 2012; Yudin and Money 2017).

Replenishment of *lactobacilli* in this condition has been suggested to restore the natural microflora homeostasis in the host body (Fig. 8.1). Clinical studies have shown that consumption of probiotics has not only helped with the prevention of BV itself but also prevents its recurrence, whereas no such compelling observations were made in case of sole antibiotic treatments (Ya et al. 2010; Hensel et al. 2011; Vujic et al. 2013). Recent meta-analyses studies exploring the use of probiotics in BV have propounded certain backlogs such as the effectiveness in BV treatments being strain specific. Lack of sufficient evidence and studies with a smaller sample size are also some of the points discussed by some research teams that make it difficult to bring probiotics fully into the treatment scenario. Strus et al. mentioned the need of a larger

study sample to better deliver their results. The study conducted by Robert Barrons and colleague also find inconsistent probiotic activities which is attributed to the small sample size and specific-strain activities, which requires further investigation as well as dose validation (Barrons and Tassone 2008). Nevertheless, the use of probiotics alone as well as in combination with antibiotics in such subjects has shown improvements, even in the vaginal pH (Strus et al. 2012; Sgibnev and Kremleva 2020). Therefore, discussing such studies, exploring the updates and advancements as well as further research is required to prove this approach as an appropriate alternative for the treatment of BV.

8.2 Prevalence and Diagnosis

The prevalence of BV on an average is about 30–50% across the world, varying in different communities and regions, of which more than 50% are asymptomatic and mostly occur in women of reproductive age. The manifested reason for the infection is the change in vaginal environment due to the disturbance of natural microflora, which mainly consists of bacteria from the *lactobacillus* species. The vaginal environment undergoes massive changes throughout the menstrual age and is therefore prone to undergo *dysbiosis*, especially during the menstrual cycles. This is mainly due to pH fluctuations throughout the menstrual cycle and frequent shedding of the vaginal epithelium. The health risks involved in BV include pelvic inflammatory disease, chorioamnionitis, and pregnancy related complications like postpartum endometritis, miscarriage, preterm delivery, and failure of procedures like in vitro fertilization (Vujic et al. 2013; Hay 2014; Yudin and Money 2017).

Dysbiosis or Dysbacteriosis is often used to describe a condition of microbial imbalance or impaired microbiota inside the body.

The most visible and symptomatic infection is identified by a set of characteristics defined as Amsel's criteria. This is considered as a *gold standard* for clinical diagnosis of BV and is characterized by:

1. Homogenous, white vaginal discharge,
2. Vaginal pH higher than 4.5,
3. Detection of *clue cells* (described by many as vaginal epithelial cells with a heavy coating of pathogenic bacteria, which could be a biofilm),
4. Fishy, amine odour on addition of potassium hydroxide (*positive whiff test*).

Validation of this diagnosis requires further microbial tests in a laboratory. Carol Spiegal and colleagues at the University of Washington first reported the use of Gram-staining for characterizing the bacteria isolated from patients having BV. Initially, pap smears and wet mounts were used for clinical diagnosis, but

these were often inaccurate in terms of visualization and identification of microorganism. The only other way to characterize was using sophisticated techniques like Gas-Liquid Chromatography which would not be easily accessible to clinicians all the time. Pap smears and wet mounts are still used in many countries, at least as the preliminary diagnostic test due to their low costs (Spiegel et al. 1983; Anand et al. 2020).

The Bethesda system (TBS), is a system that evaluates Pap smears to report cervicovaginal cytological diagnoses like cancers.

Gram-staining is still a widely used method to diagnose BV, unbiased on the microorganism characteristics and is validated to give more than 96% sensitivity, when it comes to clinical practices. However, identification of the Gram-stained microorganisms also needs expertise involves complications and differences in expert opinions (Ison and Hay 2002). Therefore, the characterized samples are further subjected to morphological and biochemical tests. These tests mainly look to either identify the pathogenic bacteria or score the depletion of *lactobacilli* in the smear. A common test to score is the Nugent's 10-point scoring system developed by Robert Nugent and his team. The scale indicates 0–3 as normal, 4–6 as intermediate, and diagnosed as BV if the score is 7 and above. Biochemical tests like chromatography, proline aminopeptidase test, etc. look at the metabolic by-products of the microflora to identify them, while advanced tests like 12 s rDNA sequencing, PCR, FISH, etc. look at the molecular identification of the pathogenic microorganisms which are efficient and overcome the challenges posed by the conventional diagnostic tests (Nugent et al. 1991; Strus et al. 2012; Vujic et al. 2013; Hay 2014; Yudin and Money 2017; Schwebke et al. 2020).

Commercial test kits including pH-paper coated vaginal swabs and pH alteration kits, rapid test kits to detect increased levels of vaginal sialidase activity like BV Blue, different PCR panels, and PCR based test kits like BD Affirm, BD Max, etc. have been in use recently. A report submitted to the US department of Health and Human Services commends the higher sensitivity of the test kits when compared to Amsel's criteria or Nugent's score alone. The report also addresses the need for publicly available rapid test kits as well as further studies to consistently show the prevalence and effective treatment in large populations, especially in pregnant women and the effect of BV on preterm delivery, post-partum health as well as the newly born child's health (Kahwati et al. 2020a, b). Due to a high demand for molecular test kits, many studies have individually evaluated and compared them, to look for the efficient one, that can detect mild and asymptomatic cases as well. MAX VP, a BD MAX vaginal panel is a real-time PCR based kit that algorithmically detects *lactobacilli* and BV-associated bacteria, show better specificity, as high as 96.1% as compared to tests like BD Affirm which detects *G. vaginalis* alone (Thompson et al. 2020).

Molecular studies have also shown microbial imbalance in non-BV or asymptomatic women as well. It is to be noted that the asymptomatic case here, is defined by lack of characteristics as described by Amsel's criteria. This could suggest that the infection is in progression and has not reached the severity to show these symptoms. Some studies suggest that these asymptomatic infections might cause severe epithelial barrier disruption and shedding in vagina and make it highly susceptible to other infections than in the symptomatic ones (Hoang et al. 2020; O'Hanlon et al. 2020). This can also be observed in some postmenopausal women and even though such observations are not made in pubescent girls, molecular testing might paint a clear picture as to the susceptibility of the infection and is therefore, very crucial.

8.3 The Microflora

8.3.1 Normal Microbiota

The normal microflora of vagina mainly consists of bacteria belonging to the phylum *Firmicutes*, sustaining in a type of *driver-passenger niche*. The predominant bacterial species or the *driver* of the niche is the *lactobacillus* or the lactic acid-producing species which maintains the microbial homeostasis. The vaginal microflora population is lesser than that of gut microflora and the *lactobacilli* is said to move from gut to vagina through the intestinal tract. Species like *L. crispatus* and *L. iners* are abundant in healthy vaginal environment, although the population of *L. iners* peaks when the environment undergoes a change and is often regarded as a marker species for the same.

Viable but non-culturable (VBNC) or uncultivable/unculturable bacteria are the ones that are not capable of growing under laboratory conditions and thus cannot be characterised and yield very little information. These are subjected to whole genome sequencing to understand and study.

8.3.2 BV Microbiota

Many factors cause the depletion of *lactobacilli* population in the vagina which leads to the overgrowth of pathogenic microbes and disruption of the microbiota homeostasis. This is also observed in the case of BV, but the overgrowth is credited to multiple microbial species rather than a single pathogen, and hence BV is known as a *polymicrobial disease* (Fig. 8.2). Phylogenetic studies suggest these BV-associated *pathobionts* to be anaerobic or microaerophiles and include species of the phyla *Bacteroidetes*, *Actinobacteria,* and *Fusobacteria.* Most commonly reported species include *Gardnerella vaginalis, Atopobium vaginae, Mobiluncus species, Bacteroides, Prevotella species, Clostridiales species, Leptotrichia species,*

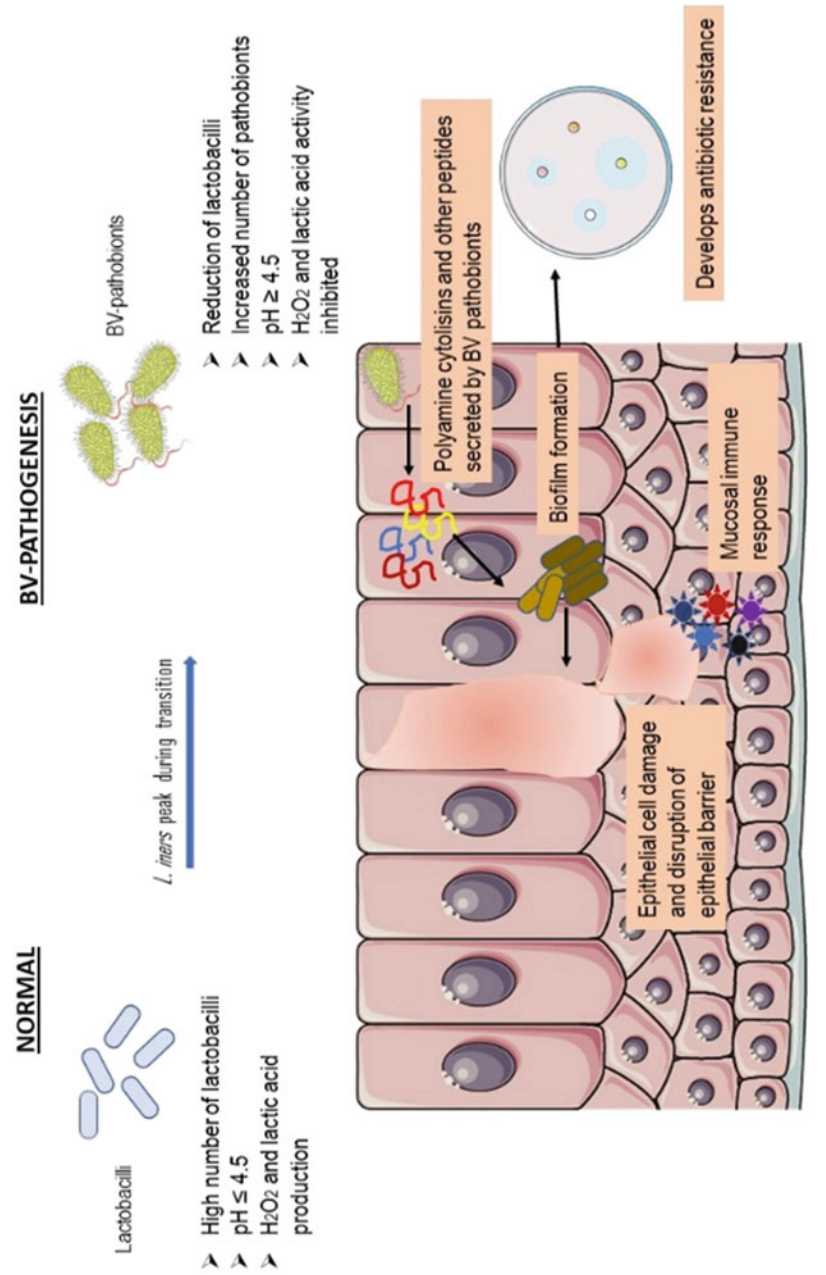

Fig. 8.2 Animation of BV pathogenesis

Megasphaera species, Eggerthella-like species, and Mycoplasma species. Of these, *G. vaginalis* which is also present in normal vaginal environment overgrows in BV and is said to be the major contributor of virulence and take part in biofilm formation *A. vaginae* is also one of the major pathogens of BV and is found in most of the reported cases along with species like *L. amnionii, A. christensenii, D. micraerophilus,* and *P. Timonensis* (Ling et al. 2010; Zozaya-Hinchliffe et al. 2010; Nelson et al. 2012; Srinivasan et al. 2012; Strus et al. 2012; Mastromarino et al. 2013; Vujic et al. 2013; Onderdonk et al. 2016; Anukam et al. 2020; Neal et al. 2020). David Fredricks and team at the Fred Hutchinson Cancer Research Center carried out rDNA sequencing and PCR analysis of vaginal samples, which detected three new bacteria in BV samples, apart from the other majorly reported ones. They named these *Bacterial Vaginosis-associated bacteria* (BVAB) 1, 2, and 3 and had phylogenetic relation to the phylum *Clostridium*. These bacteria are *non-culturable* and so very less is known about them, nevertheless, they have been reported in other studies by various researchers. A recent 16s RNA sequence study identifies BVAB1 as a member of the family *Lachnospiraceae* and the closest relative of *S. satelles*. They propose *Candidatus lachnocurva vaginae* as a candidate species. The sequencing gave critical information about the suggestive pathogenic factors that show the capacity of BVAB1 producing haemolysin, active drug efflux pumps and antibiotic resistance mechanisms along with the possibility of flagellar motility. A plausible choline mediated Trimethylamine production can be attributed to fishy odour which is characteristic to BV (Fredricks et al. 2005; Holm et al. 2020).

Pathobionts are pathogens that co-exist harmlessly under normal conditions. The BV associated pathogens are also present in the normal vaginal microflora, but their population is kept in check by other species like lactobacilli. They cause infections only during dysbiosis.

8.3.3 Biofilms

It has been suggested that a predominant BV-associated bacterium like *G. vaginalis* first colonizes to form strong attachments to the vaginal epithelium, sort of like a *driver* species in this scenario, and subsequently facilitates the attachment of other bacteria, thereby forming close synergism and developing biofilms. *A. vaginae* has often been detected in major *G. vaginalis* biofilms and are believed to be in strong association with each other. Studies have shown that in these associations only certain strains of *G. vaginalis* provides a scaffolding structure while *A. vaginae* does not provide such scaffoldings but only attaches as an *associate* organism and benefits from *G. vaginalis,* which in turn strengthens the biofilm and its virulence. 16s rDNA analyses and FISH visualizations of BV affected tissues have shown the association of biofilms mainly of *G. vaginalis* in the vagina, as well as in the uterine endometrium thereby suggesting the strong spread and colonization of BV microflora through the biofilms and could ultimately be responsible for the BV-associated pregnancy complications. BV-associated biofilms have also been known to provide

attachment for other parasites and increase the susceptibility to acquire other urinogenital and sexually transmitted infections. These biofilms have been known to develop resistance towards antibiotics and cause severe infections and recurrences. Certain species like those belonging to the genus *Mobiluncus* have shown major resistance to antibiotics like metronidazole. A review by Verstraelen and Swidsinski draws this conclusion by comparing it with the process of *coaggregation* in oral infections. Upon treatment with metronidazole, these biofilms were temporarily suppressed and went dormant but regained activity after the treatment was stopped. Octenidine has been shown to eradicate polymicrobial biofilms, but show recurrence in the long run, thereby suggesting that antibiotic treatment alone might not be sufficient to treat BV, at least those involving biofilms and the use of probiotics might replenish *lactobacilli* and eventually outgrow the BV-associated microflora. This sort of combined treatment seems to be more practical as biofilms of *G. vaginalis* are reported to be resistant to lactic acid and hydrogen peroxide and would sustain in a sole probiotic treatment (Swidsinski et al. 2013; Verstraelen and Swidsinski 2013; Onderdonk et al. 2016; Castro et al. 2020b; Hinderfeld and Simoes-Barbosa 2020).

Coaggregation is a process where a single species first adheres to a tissue and poses as a scaffold for the adherence of other species.

8.3.4 Vaginal Homeostasis

Vaginal homeostasis is very important as it dictates the vaginal hostility towards the pathogenic microflora. The pH around 4.5 allows the efficient activity of lactic acid and hydrogen peroxide. Hydrogen peroxide plays an important role of initiating oxidative stress in microbial cells, and lactic acids acts by permeating the cell membrane, thereby inducing osmotic stress in the microbial cells while *lactobacilli* present in the vaginal microflora develop mechanisms to protect themselves from these acids. This effect of the *lactobacilli* acids is nullified at pH 7, and is thus ineffective in killing pathogenic microorganisms. However, it is also observed in some studies that hydrogen peroxide has no significant effect against pathogenic microbes in vitro and in vivo, whereas the action of lactic acid holds true in both the cases. Some studies suggest the change in pH due to sexual activity and menstrual cycle could inhibit lactic acid and hydrogen peroxide activities, that would in turn fail to stop the invasion by pathogenic polymicrobial species. Another obvious reason or trigger point would be a depletion in the *lactobacilli* population itself, which could be due to certain bacteriophages or other bactericidal/bacteriostatic factors. There have been indications of the involvement of lysogenic phages of *lactobacilli* that contribute towards BV as they enter into their lytic cycle (O'Hanlon et al. 2011; Nelson et al. 2012; Srinivasan et al. 2012; Castro et al. 2020a). Given these interventions, balancing the conditions of vaginal environment is crucial and is driven by pH fluctuations. Once the pH increases, the population density shifts from *lactobacilli* towards the pathobionts which further acts on the vaginal epithelium.

Recent studies explain that the microbiota inhabits the vaginal squamous epithelium. This epithelial layer undergoes a cycle of proliferation, maturation, and shedding. When energy is required, the glycogen in vaginal epithelium undergoes fermentation by the microbiota to sequentially form glucose, pyruvate, and lactic acid; while that present in the vaginal lumen ferments to form lactic acid by the lactic dehydrogenase produced by the *lactobacilli* in the vaginal microbiota. This lactic acid production creates a favourable environment for *lactobacilli* and other species in the normal environment while providing defence against pathogenic species. The *lactobacilli* in the vaginal environment are not only known to produce lactic acid, hydrogen peroxide and maintain the acidic pH, but also produce antimicrobials like bacteriocins, ligands, etc. BV-associated bacteria on the other hand, produce certain metabolites and enzymes like phospholipase C and urease, that hydrolyse urea to form cytotoxic ammonia. This affects the pH and hinders the activity of lactic acid by neutralizing it which will trigger dysbiosis.

Polyamine cytolysins secreted by BV-associated pathobionts such as vaginolysin, sialidase, glycosulfatase, collagenase, fibrinolysins, etc. destroy the vaginal cells and mucin, and increase the pH thereby altering the vaginal environment. *G. vaginalis* and *Mycoplasma* secrete adhesins and similar compounds to facilitate their adherence to the vaginal tissue. This alteration facilitates a favourable attachment and breeding site for the polymicrobial niche, even to the extent of forming biofilms. The characteristic fishy odour of vaginal discharge is suggested to be due to the bacterial polyamine secretions like putrescine, cadaverine, trimethylamine, etc. (O'Hanlon et al. 2011; Nelson et al. 2012; Srinivasan et al. 2012; Onderdonk et al. 2016; Barrientos-Durán et al. 2020). A study shows that vaginolysin produced by *G. vaginalis* and inerolysin produced by *L. iners,* belonging to the pore-forming toxin family are cholesterol dependent and bind to the human complement glycoprotein CD59 and induce cellular damage. Vaginolysin is active in the pH range for BV and induces damage and increases epithelial permeability by disrupting the vaginal epithelium and endothelium as well as host immune response. However, inerolysin is active in the pH range 4.5–6.0 which is seen in the normal vaginal environment and is believed to help the survival and adaptation of *L. iners* itself in the changing vaginal environment. A study suggests that vaginolysin also affects brain cells and cause encephalopathy by penetrating the blood–brain barrier (Pleckaityte 2020). Another study shows caspase-3 activation in *G. vaginalis* directed BV samples which induce apoptosis in the vaginal epithelial cells (Roselletti et al. 2020).

8.4 Immune Response in BV

The uptake of *G. vaginalis* by the vaginal epithelium is reported to induce cytoskeleton reorganization in the epithelium, thereby weakening the epithelial barrier. This triggers the mucosal immune response in vagina that activates immune factors. In vitro studies have shown upregulation of proinflammatory markers like IL-1α, IL-1β, IL-6, IL-8, IL-12p70, IFN-γ, TNF-α, NF-κB, GM-CSF, RANTES, SLPI,

IP-10, MIP-3α and antimicrobial peptides like hBD-2 upon infection of cells with BV-associated bacteria like *G. vaginalis* and *A. vaginae*. *Megasphaera elsdenii* and *Prevotella timonensis* induce genital inflammation through dendritic cells and overexpress CD80, CD83, and CD86. They induce the production of inflammatory markers IL-1β, IL-6, IL-2, IL-8, and TNFα. In vivo studies show that certain pore-forming toxins like inerolysin and vaginolysin activate p38MAPK and upregulate proinflammatory cytokines and IgA. The pathogens also suppress such mucosal immune response by secreting proteases and elastases that destroy IgA. This might decrease or show no inflammation in the lower genital tract but upregulate the same in the upper genital tract. This leads to conditions like PID and cervicitis, which is characteristic to BV. The fact that these markers are also expressed during HIV infection, suggests the susceptibility of acquiring STIs in BV cases. The cervicovaginal mucus poses as a barrier and has been seen to consistently defend against HIV in healthy vagina, whereas this barrier failed to stop the influx of HIV virions in that of BV cases. The proinflammatory markers are also elevated in women predicted with preterm delivery and adding this to the fact that they are believed to translocate and move from vaginal tract to uterus, it suggests a strong association of BV with preterm labour and other pregnancy complications too (Onderdonk et al. 2016; Hoang et al. 2020; Muzny et al. 2020; Pleckaityte 2020; van Teijlingen et al. 2020).

Rantes, also known as *CCL5* (*CHemokine Ligand 5*) is a chemokine expressed in HIV infections and is considered as a predictive marker of HIV and is a major target of anti-HIV drugs.

hBD-2 or human beta defensin 2 is an antimicrobial peptide in epithelial cells and is secreted as an immune response to microbes.

8.5 Factors Associated with BV

8.5.1 Menstrual Cycle and Reproductive Age

The vaginal environment undergoes various changes throughout the reproductive age, which only makes it obvious for the associated microbiota to witness some imbalances. The predominance of various species in the vaginal microflora changes at different reproductive stages. For instance, *Lactobacillus, Atopobium,* and *Streptococcus* species are dominant during puberty and the population of *lactobacillus* decreases in postmenopausal women. This decrease is often attributed to depleted oestrogen levels and the consequent pH change that may facilitate the colonization of pathogenic microbes. The menstrual cycles itself sees changes in hormones, pH, etc. which makes the urinogenital niche susceptible to dysbiosis. Vaginal microflora of pregnant women is stable in comparison with that of non-pregnant women due to high levels of oestrogen present in the body during this period. However, there is

considerable dysbiosis of vaginal microflora in some, which increases the susceptibility to BV and other urinogenital infections. The high amount of glycogen present during the gestational period may enhance the growth of *lactobacilli* in the vagina and thus may be responsible for maintaining the vaginal microbial homeostasis in that period. The levels of *G. vaginalis* and *L. iners* increase during menstrual cycles and decrease at the end of menstruation. Some PCR based studies suggest that *G. vaginalis* needs iron for growth, which is plenty available in the vagina during menstruation. Laboratory cultures of *G. vaginalis* on blood agar medium and its inability to grow in iron-limiting media support this claim. As for *L. iners,* as mentioned in the previous section, it may peak due to the changes in the vaginal environment and serve as a marker to track this (Srinivasan et al. 2010; Barrientos-Durán et al. 2020; Kalia et al. 2020; Sule-odu et al. 2020).

8.5.2 Sexual Activity, Sexually Transmitted Infections (STI), and Other Urinogenital Infections

Sexual intercourse even at a younger age has been associated with increased risk of BV contraction. Sexual activities in general might cause change in the vaginal pH which might result in a change of microflora or even dysbiosis. The BV-associated pathogens might also be transmitted from females to males or vice versa, although there are no reports of severe infections or studies carried out in the former case, and are more likely to occur only if the vagina is infected with dense biofilms of BV pathogens. Some studies suggest that the penile microbiome has the tendency to induce BV in women after sexual activities and can be prevented by using condoms, but is not conclusively confirmed due to the lack of studies in this aspect. An Indian study claims that women having STI have at least two times the risk of contracting BV and vice versa (Srinivasan et al. 2010; Yudin and Money 2017; Castro et al. 2020a; Joshi et al. 2020; Neal et al. 2020).

It has been posed by many studies that BV infections create the favourable environment for viruses causing STI to replicate faster which, along with the vaginal shedding observed in BV allows the easier spread of STI viruses and hence may be the reason why both STI and BV are associated with each other (Verstraelen et al. 2010). Therefore, many studies suggest the use of the term "sexually enhanced disease" to describe BV and its association with sexual activities (Nelson et al. 2012). Brandie Taylor and colleagues from the University of Pittsburgh have reviewed the relation of BV and Pelvic Inflammatory Disease (PID), and found similar contrasting reports, which nevertheless suggest that poor immunity due to BV does make the patient susceptible to other infections like PID (Taylor et al. 2013). A prior meta-analysis done with the data available at that time, also suggested that the association of BV and HPV (human papilloma virus) infection to be controversial, although BV provided a site for its easy acquisition (Gillet et al. 2011).

Recent studies, however, have found more compelling evidences that associate BV with STI and other urinogenital infections. BV has been conclusively associated with a higher risk of HPV by displaying abnormal cervical cytology (Li et al. 2020)

and have shown susceptibility to microorganisms like *Neisseria gonorrhoeae*, *Trichomonas vaginalis, Chlamydia trachomatis, Candida*, and even HPV, HSV-2, and HIV-1, thereby increasing the risk to contract diseases caused by these pathogens (Ling et al. 2010). *C. trachomatis*, *M. genitalium*, and *T. vaginalis* infections have been observed in BV affected women having a lower risk of STI, irrespective of their age (Shipitsyna et al. 2020). Infections caused by *M. genitalium* have been recorded to be significantly higher in women with BV (Nye et al. 2020). This susceptibility of viruses and other pathogens is due to the disrupted host epithelial barrier. It has been observed that BV-associated pathobionts trigger an increase in the host matrix metalloproteinases that disrupt the endocervical epithelium and increase facilitate the attachment and growth of other viruses and pathogens (Cherne et al. 2020).

8.5.3 Ethnicity and Demographic Diversity

Microbial diversity is reported to be varied across the ethnic and demographic populations, and may be related to the genetic make-up of the women themselves. Many reports and clinical studies have found the prevalence of BV in African and black women, as well as in ethnic groups of particular regions. When the vaginal samples of Black women were compared with that of White women in a PCR based study, it was found that bacteria like *L. amnionii, A. vaginae,* and BVAB1 were predominantly found in Black women, and the microbial diversity in vagina is seen in Black and Hispanic women, which include the BV-associated species (Srinivasan et al. 2012). Most of the BV studies across the demographic regions show different prevalence in terms of infection rates, that were found to be higher in Hispanic and African women whereas White and Asian women have a higher population of *lactobacilli* (Onderdonk et al. 2016; Soper 2020). A study evaluating prevalence of *M. genitalium* infections in BV women shows a strong influence of ethnicity and age, as it mentions African-American women of age below 25 years. Major factors studied in this aspect have been ethnicity and socio-economic status, although these might not necessarily be the only factors for the disease prevalence (Nye et al. 2020).

8.5.4 Other Factors

Some studies suggest that a deficiency of Vitamin D may be a risk factor of BV, predominantly in pregnant women but is also seen in non-pregnant women. It was also studied that smoking could be a potential contributor of the disease in non-pregnant women (Hensel et al. 2011). Stress has also been associated with BV, as the hormonal changes result in glycogen level fluctuations. As discussed initially in this chapter, glycogen is required for lactic acid production which helps *lactobacilli* and keeps the microbial homeostasis in check. Therefore, if glycogen levels drop, it could decrease the levels of lactic acid and therefore increase the vaginal pH (Barrientos-Durán et al. 2020). Infection and sepsis during surgeries is a

common knowledge and has been observed in gynaecological operations as well. Women having even mild cases of BV have shown increased severity after surgeries like caesarean delivery, hysterectomy, early pregnancy and abortion operations, etc. Diagnosis and antibiotic administration prior or during these surgeries have shown lesser rates of postoperative BV infections (Soper 2020). Vaginal douching is also one of the risk factors of BV with a 20% increased incidence rate (Wan and Jacobs 2018). This has been attributed to the dysbiosis resulting after the douching activities and inflammation due to irritation. This has been associated with increased susceptibility to STI as well (Brotman 2008).

8.6 Treatment

8.6.1 Conventional Antibiotic Treatment

Women with BV are conventionally prescribed antibiotics like metronidazole, tinidazole, and clindamycin. The recommended antibiotic treatment for BV in terms of oral administration is 500 mg Metronidazole or 300 mg of Clindamycin, twice daily for 7 days. 2 g of tinidazole one time for 2 days or 1 g for 5 days is also recommended by CDC (Centre for Disease Control and Prevention). Other administrations include 5 g of clindamycin vaginal creams for 7 days or 100 mg of clindamycin intravaginal ovules for 3 days and 5 g of metronidazole intravaginal gel for 5 days. Some randomized studies suggest that clindamycin creams are an effective alternative for oral metronidazole although there is no significant difference in their treatment rates (Schmitt et al. 1992; Yudin and Money 2017; Soper 2020).

These antibiotics have reported side-effects like gastrointestinal disturbances, metallic taste in mouth, nausea, paresthesia, leucopenia, sensitivity reactions, pseudomembranous colitis, etc. and deteriorating effectiveness. Combinations as well as newer antibiotics are being explored lately to overcome these. Metronidazole has been paired up with wide-spectrum antibiotics like erythromycin to enhance the antibiotic activity. Secnidazole is a new antibiotic that has been gaining a lot of attention (Brocklehurst et al. 2013; Yudin and Money 2017; Soper 2020). Treatments with secnidazole have significantly reduced the BV symptoms in women, in some cases better than the conventional antibiotics. Even microgranule formulations have proven effective than the regular FDA approved dosage. These may comprise of 1 or 2 g of secnidazole with sugar spheres, PEG, silicon dioxide in colloidal form, etc. (Pentikis et al. 2020).

The main idea of antibiotic intervention is to reduce the growth of abnormal microflora, thereby facilitating the growth of *lactobacilli* and other normal microbiota to restore vaginal homeostasis. But complete eradication of pathogens is not possible due to many factors and this leads to recurrence of infection. One of the major of the major reasons of treatment failure is antibiotic resistance. Moreover, even successful treatments have failed to ameliorate the associated pregnancy complications.

8.6.1.1 Antibiotic Resistance and Failure to Reduce Pregnancy Complications

There is an increased incidence of antibiotic resistance, attributed mainly to the presence of biofilms which also leads to recurrence of BV. The recurrence rates, which go anywhere from 15 to 60% suggest that the environment is still hostile for adequate *lactobacilli* growth (Ehrström et al. 2010). The biofilms have a strong adherence and association which aggravates the infection. Due to this, antibiotic administration is often extended than the usual recommendation of 5–7 days. This prolonged exposure of abnormal microflora to antibiotics induces resistance in them. A randomized clinical trial shows intravaginal clindamycin are more prone to antibiotic resistance than metronidazole treatments (Beigi et al. 2004). This is due to direct and prolonged exposure of the BV microflora to the antibiotic formulation. Another complication is the infection in upper genital areas or extra-vaginal areas. BV infections in the upper genital tract cannot be treated with vaginal creams and gels. Even if such extra-vaginal infections persists in women, only oral administration would be effective rather than the intravaginal ones and failure to eradicate microflora in this area might also lead to recurrence (Fredricks et al. 2020). Other studies however, show failure of oral antibiotics in the scenario of antibiotic resistance in the niche itself. To overcome this, many administrations are being changed to combinations with wide-spectrum antibiotics and novel formulations, even so there is a lack of attention in pregnant women. Treatment with clindamycin vaginal creams have been known to ameliorate the BV infections but failed to reduce pregnancy complications like preterm delivery and low birth weight. Similar results have been observed in other antibiotic administrations which also failed to reduce preterm membrane ruptures as well as neonatal sepsis and postpartum infections. Although some reports do show a significant decrease in preterm births, there is no clarity as to the rate of risk and severity of BV in these cases (Joesoef et al. 1995; Brocklehurst et al. 2013; Mcdonald et al. 2014).

8.6.2 Alternate Treatment Strategies

Prior to antibiotic administration, vaginal gels having boric acid were used to reduce the outgrowth of abnormal microbes. More recently, they have been modified with EDTA incorporation to target biofilms. However, a modern class of compounds like dendrimers are increasingly gaining the attention in drug delivery and targeting. The dendrimers themselves are capable of targeting and disrupting biofilms. Astrodimer (1%) gel formulations have shown better efficacy when compared to metronidazole, secnidazole and even reduce the foul odour (Waldbaum et al. 2020). Vaginal gels and antimicrobial monoglycerides like monolaurin have been explored and remained ineffective in treating BV cases (Mancuso et al. 2020). Since BV mechanism involves cholesterol-dependent cytolysins like vaginolysin, drugs like statins have also been an attractive alternate for BV treatment. The drug reduces the cholesterol in the vaginal epithelium thereby reducing vaginolysin activity and providing lesser chance of growth for *G. vaginolysin* and its biofilms. Intravaginal ezetimibe

administration through vaginal rings alone or with statin also induces similar effects and have been explored as carriers (Jefferson et al. 2020).

Destruction or eradication of the pathogenic bacterial strains from the host body does not necessarily lead to complete treatment, as the vaginal environment is still hostile and can undergo a relapse. The pH can be temporarily adjusted, but in the long run can go back to alkaline pH, and might again attract the pathogenic microbes. The plausible solution for this seems to be the replenishment of *lactobacilli* in the vaginal environment and thus restoration of its homeostasis. This is established through external supplementation of *lactobacilli* through the administration of probiotics, which has been reported in many studies since the 1990s. Consumption of *lactobacillus*-rich food like yoghurt have shown a significant increase in their colonization in the rectum and vagina. Similar observations have been reported after the consumption of probiotics containing *lactobacillus* species. Yeast based foods are also gaining importance in fermented foods and probiotics as strains like *Saccharomyces* CNCM I-3856 show significant activity against *G. vaginalis.* They reduce the bacterial load by clearing up to 90% of the species ultimately reducing sialidase activity and epithelial cell damage in vagina (Gaziano et al. 2020). Antibiotic alternatives like antimicrobial formulations are also being explored to treat biofilm induced resistance and recurrence, and seem like good candidates for probiotic based combination therapies as well. Therefore, a more plausible remedy in the wake of antibiotic resistance and biofilm associated infections, would be to replenish the *lactobacilli* population and restore the vaginal homeostasis.

8.6.3 Probiotic Administration Modulates BV Niche and Might Reduce Recurrence Rates

Probiotics have been an attractive approach in itself as well as in combination with the use of antibiotics, as they have negligible side effects and clearly addresses the problem of recurrence. Although the *lactobacilli* replenishment in the vagina is subjective to their attachment to the epithelium, and has not always been 100% successful, its administration has shown improvement in terms pH, vaginal environment, modulation of the inflammatory markers and immune response, with the control of pathobiont growth thereby restoring the vaginal homeostasis (list of probiotics strains and their activities mentioned in Table 8.1). Therefore, this seems to be a better approach even for asymptomatic or mild cases of BV.

Lactobacillus strains like *L. rhamnosus* GR-1 and *L. fermentum* RC-14 have been extensively studied for BV treatment as they exhibit effective adhesion to vaginal epithelium and inhibit the growth of pathogenic bacteria through the production of hydrogen peroxide and other compounds (Vujic et al. 2013). *Lactobacillus brevis* CD2, *Lactobacillus salivarius* FV2, and *Lactobacillus plantarum* FV9 also exhibit these properties and reduce the expression of proinflammatory markers and reduced inhibitory activity towards STI virus strains like HSV-2. Though these clinical studies showed varying rates, they showed effective treatment of BV and upon

Table 8.1 List of the discussed probiotic strains and their activities in the BV scenario

Probiotic strain	Activity
L. rhamnosus GR-1	Antimicrobial, improves vaginal microflora—adheres to epithelial cells and competitively inhibits epithelial adherence of pathobionts, produce H_2O_2 and other compounds
L. rhamnosus BPL005	Antimicrobial, reduce the vaginal pH, reduce BV relapse in combination with antibiotics
L.fermentum RC-14	Antimicrobial, improves vaginal microflora—adheres to epithelial cells and competitively inhibits epithelial adherence of pathobionts, produce H_2O_2 and other compounds
L. brevis CD2	Antimicrobial and immunomodulatory—inhibit proinflammatory markers, act against viruses like HSV-2
L. salivarius FV2	Antimicrobial, inhibits *G. vaginalis,* produces H_2O_2 and immunomodulatory—inhibit proinflammatory markers, act against viruses like HSV-2
L. plantarum FV9	Antimicrobial and immunomodulatory—inhibit proinflammatory markers, act against viruses like HSV-2
L. gasseri 57C	Antimicrobial, prevents clinical relapse of BV, reduce the vaginal pH
L. gasseri LN40	Replenishment of vaginal *lactobacilli,* amelioration of pathogenesis
L. gasseri 335	Inhibits *G. vaginalis*, produces H_2O_2
L. acidophilus 48101	Inhibits *G. vaginalis, P. bivia, Bacteroides*; produces H_2O_2, reduces BV recurrence
L. casei LN113	Replenishment of vaginal *lactobacilli,* amelioration of pathogenesis
P. acidilactici	Replenishment of vaginal *lactobacilli,* amelioration of pathogenesis
S. thermophilus	Reduces BV recurrence
L. pentosus KCAI	Produces biosurfactants, lactic acid and H2O2, inhibits growth of pathogens, known to disrupt *G. vaginalis* biofilms, modulates inflammatory pathway

evaluation with Nugent's score, indicated on the scale with ≤ 3. Molecular studies revealed that the probiotic enhanced vaginal health by upregulating Menaquinone biosynthesis pathway, Vitamin B6 and downregulated IL-1β. A placebo-controlled clinical trial involved treatment with a cocktail of *lactobacillus* strains *L. fermentum* 57A, *L. plantarum* 57B, and *L. gasseri* 57C showed improvements in BV and *Aerobic vaginits* cases while delaying their clinical relapse, as a result of the *lactobacilli* replenishment and the consequent decrease in pH (Hallen et al. 1992; Strus et al. 2012; Mastromarino et al. 2013; Heczko et al. 2015). A patented strain of *L. rhamnosus,* BPL005 also has similar pH reducing activity and claims to be better than other probiotics as it maintains its efficacy even after large-scale productions. The strain in probiotic formulations seems to be attractive for both pharmaceutical and dietary applications. Even more efficient results were seen in cases of combined treatment with the *lactobacilli* and antibiotics, and reduced the relapse of BV by 76% (Chenoll Cuadros et al. 2020). A recently published pilot study administered commercially available probiotic *lactobacilli*-Ecologic Femi+ vaginal capsule that has a cocktail of lyophilized *B. bifidum* W28, *L. acidophilus* W70 and *L. helveticus* W74, *L. brevis* W36, *L. plantarum* W21, and *L. salivarius* W24; along with the tablet Gynophilus LP that has *L. rehamnosus* 35. Although the researchers found

inconclusive results, they did mention that using probiotics may reduce BV recurrence and boost the natural *lactobacilli* population in the host vaginal environment, thereby enhancing the lactic acid production, vaginal immune response and inhibit the formation of pathogenic biofilms (van de Wijgert et al. 2020). A study conducted in Nigeria shows the oral administration of *Lactobacillus pentosus* KCA1 in 7 women with BV decreases BV-associated bacteria and disrupted the biofilm, while increased the growth of normal vaginal microflora (Anukam et al. 2020).

Aerobic vaginitis is a condition of *intermediate flora* when aerobic bacteria outgrows lactobacilli.

8.7 Conclusion

Bacterial vaginosis is a common infection in women with varying prevalence across ethnicities, and is a growing concern due to its associations with sexually transmitted infections and pregnancy complications. The antibiotic treatment is a widely used and universally recommended solution. The antibiotics only induce bactericidal or bacteriostatic effects against the pathogens and so it is obvious that they would have any effects to ameliorate pregnancy complications. The preterm membrane rupture and delivery is believed to be due to epithelial cell damage and shedding in the vagina. It is now known that the pathogens secrete polyamines that induce such cellular damage and is only aggravated due to biofilms. The biofilms also facilitate the attachment of other pathogens and this is where the susceptibility factor to STI and other urinogenital infections comes in. All these processes induce mucosal immunity in the host vagina which fights these pathogens. But it is also known that the biofilms can suppress this immune response as well. This only worsens the dysbiosis and infection. To diagnose this condition, it is important to establish good molecular tests that are not only sensitive and efficient, but also accessible to all. A survey-based study in postmenopausal diagnosis and treatment of BV remarks a lack of uniformity and the need to further develop molecular tests for diagnosis. Due to many cases being asymptomatic and mostly varying from the usual BV cases, many clinicians prefer to rely on molecular tests in postmenopausal patients (Mark et al. 2020). Hence, it is important to focus on this aspect and develop further test kits and systems to identify susceptibility in asymptomatic patients as well. This may prove to be helpful in providing better prognosis and treatment for women already affected with urinogenital infections. It is also handy for those undergoing surgeries as it would reduce the risk of postoperative infections as well as postpartum complications.

So far as the diagnostics go, conventional antibiotic treatment is also a major concern as we have been seeing antibiotic drug-resistance in general. Even when we come up with new antibiotics, it is only a matter of time to face these problems all over again. While combination antibiotics also seem to be effective, there are a lot of

strains developing multi-drug resistance and evading their action. This probably calls for a better molecular evaluation of the mechanisms involved in these processes and therefore, further research in this is very much needed. While this is at hand, probiotics seem to give a great alternative as this is nothing but replenishment of the natural microflora. As discussed, there are many advantages to its use and have proven to work efficiently alone as well as in combination with antibiotics. Intravaginal applications do certainly require the bacteria to attach to the epithelium, but its oral administration also needs to be studied further. Studies have shown that gut microbiota travel and the success rate of the probiotic supplements in terms of this needs to be studied for vaginal replenishment. Although there are enough molecular evidences to show the probiotic activity, many more studies with larger sample sizes are required to conclusively establish this as an effective treatment strategy.

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9 Indigenous Probiotic *Lactobacillus* Strains to Combat Gastric Pathogen *Helicobacter pylori*: Microbial Interference Therapy

Nabendu Debnath and Ashok Kumar Yadav

Abstract

Helicobacter pylori (*H. pylori)* infection is recognized as a causative agent for acute and chronic gastritis. In addition, infection caused by *H. pylori* often leads to the development of peptic ulcer, gastric lymphoma, and gastric cancer. Chronic *H. pylori infection* affects approximately 50% of the world's population. After infection, *H. pylori* can bind tightly to gastric epithelial cells by multiple bacterial membrane adhesins and damages gastric mucosa. An exotoxin secreted by *H. pylori* induces apoptosis of epithelial cells. *H. pylori* can also stimulate a pro-inflammatory Th1 response involving interleukin-2 (IL-2), IL-6, IL-8 as well as IL-1β and tumour necrosis factor-α (TNF-α). An attempt to treat *H. pylori* infection with antibiotics such as amoxicillin, clarithromycin, and proton pump inhibitors, however, causes serious side effects and recent success rate has also been decreased due to the emergence of antibiotic resistant *H. pylori*. Hence there is an urgent need to explore therapeutic and preventive efficacy of naturally occurring indigenous probiotic microorganisms to counter the early stages of acute infection. Several in vitro studies have reported that probiotic bacteria such as various species of *lactobacillus* or their cell-free cultures inhibit or kill *H. pylori.* Probiotics exhibit its anti-*H. pylori* activities by secreting bactericidal metabolites, decrease the production of pro-inflammatory cytokines, attenuate *H. pylori* associated hypochlorhydria, and prevent adhesion of *H. pylori* to mammalian epithelial cells. In addition, several meta-analyses have revealed the beneficial effects of probiotics when used in combination with the standard therapy. This combination increases *H. Pylori* eradication rate and decreases

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standard therapy related adverse effects. In this regard, administration of probiotic microorganisms along with triple therapy achieved a success rate against the *H. pylori* infection.

Keywords

Indigenous · Probiotics · *Lactobacillus* · *H. pylori* · antibiotic resistances · Microbial Interference Therapy

9.1 Introduction

H. pylori is a gram-negative, spiral-shaped, microaerophilic bacterium that mainly colonizes mucosal layer of the gastric epithelium and is associated with chronic gastritis, peptic ulcers, gastric adenocarcinoma and rarely, produces lymphoma in mucosa-associated lymphoid tissues (MALT) (Marshall and Warren 1984; Forman et al. 1991; Lee et al. 1997). In general, the *H. pylori* infection is predominant in developing countries than developed countries and it is very common in Indian subcontinent (Bardhan 1997). *H. pylori infection* occurs early in life and several studies have shown that 79–83% of the population is exposed in the first two decades of their life time (Graham et al. 1991; Gill et al. 1994). Serological surveys indicate that 38–40% of the children under the age of five have anti-*H. pylori* IgG/IgA in their serum and increasing up to 52–60% by the age of 20 (Kang et al. 1999; Jais and Barua 2004). *H. pylori* needs to bind to the epithelial lining of the stomach and small intestine (specifically duodenum) to induce its effects. Establishment of intimate interactions with the epithelial surface is carried out by outer membrane proteins (OMPs) such as BabA, SabA, AlpA/B, HopZ, and OipA which acts like adhesions and binds epithelium-associated lipids and carbohydrates (Backert et al. 2011). Several molecules are secreted from *H. pylori* after binding with the epithelial cells which are responsible for pathogenicity (Table 9.1). For example, urease, an enzyme breaks down urea into ammonia that eventually disrupts the cellular tight junctions and microvilli (Smoot 1997). *H. pylori* can either translocate its products such as proteases and urease through a type 4 secretion system (T4SS) encoded within the *cag* pathogenicity island (*cag* PAI) or it can secrete its products without directly interacting with the epithelium (Fig. 9.1). *H. pylori* also carries *vacA* gene which produces a protein cytotoxin that induces vacuolation in gastric epithelium. HtrA, a serine protease, is also secreted from *H. pylori* which cleaves the ectodomain of E-cadherin and disrupts epithelial barrier integrity and permits *H. pylori* to invade the intercellular space between epithelial cells (Hoy et al. 2010; Löwer et al. 2008). In addition, *H. pylori* has the capacity to induce epithelial cell apoptosis by the activation of several pro-apoptotic proteins (Bcl-2 family proteins) and protease enzymes (caspase-3, caspase-8, and caspase-9) (Shibayama et al. 2001). *H. pylori* can also stimulate cell proliferation as a compensatory mechanism which they utilize as a niche for their replication (Mimuro et al. 2007; Ashktorab et al. 2008; Olivares et al. 2005; Iwai et al. 2007). This higher level of cellular apoptosis induced by

Table 9.1 Various virulence factors of *H. pylori*

vacA	Encodes a cytotoxin protein that induces vacuolation in eukaryotic cells
cagA	Stimulates the production of interleukin-8; a part of it also codes for type IV secretion system
babA	Binds to Lewis b antigen displayed on the surface of stomach epithelial cell
iceA	Up-regulated upon contact of *H. pylori* with the gastric epithelium
oipA (*hp0638*)	Induces IL-8 secretion by epithelial cell
picB	Induces IL-8 expression in gastric epithelial cells
Urease	Neutralizes acid, also act as adhesion and maintenance factors
hp0169	Encodes for a collagenase and essential for *H. pylori* for colonization in stomach epithelium
comB4	Essential for colonization, as it encodes a putative ATPase which is a part of DNA transformation associated type-IV transport system
rocF	Encodes arginase that facilitates production of ammonia and favours nitric oxide (NO) production in stimulated macrophages
MUC5AC	Primary receptor for *H. pylori* in human stomach

H. pylori plays the significant role in the aetiology of gastritis, peptic ulcers and neoplasia, gastric adenocarcinoma, and lymphoma. Severity and risk of these physiological outcomes depend on the environmental and dietary factors, host's genetic background, specific virulence factors present in some strains of *H. pylori* such as the *Cag*PAI (Crowe 2005; Gotteland et al. 2001a), the extent of inflammation, and the density of *H. pylori* colonization, etc. (Ernst and Gold 2000a). The chances of developing peptic ulcer and gastric cancer are co-related with the increased rate of infection (Ernst and Gold 2000b; Tokunaga et al. 2000).

In India and in the world as well, incidence of *H. pylori* related chronic gastritis, duodenal and gastric ulcer is quite high (Saha 2004) and prevalence of drug resistant *H. pylori* strains has created a situation where conventional treatment is becoming ineffective. The triple therapy treatment which includes two antibiotics (clarithromycin and amoxicillin) and a proton pump inhibitor (such as Omeprazole, Lansoprazole, Pantoprazole, Rabeprazole, Esomeprazole, Leminoprazole) is being widely used in present time (Malfertheiner et al. 2007). This triple therapy is although quite successful, but responsible for many adverse effects such as diarrhoea, nausea, bloating, taste disturbance, etc. In the early 90s *H. pylori* eradication success rate with this triple therapy was more than 80% which is drastically reduced due to antibiotic resistance in recent times. In some regions where high resistance to clarithromycin is present, a quadruple therapy which includes four drugs such as tetracycline, metronidazole, bismuth subsalicylate, and proton pump inhibitor (PPI) is recommended as primary therapy (Safavi et al. 2016). If first line of therapy fails to improve the condition, second line therapy should be prescribed which should not include repeating use of metronidazole or clarithromycin (Weeks et al. 2000). If the *H. pylori* resistance is undefined, rifabutin-based triple therapy (PPI, rifabutin, and amoxicillin) for 10 days can be considered. Furthermore, other types of therapeutic regimens are also being used like quadruple therapy in which 5-day dual therapy

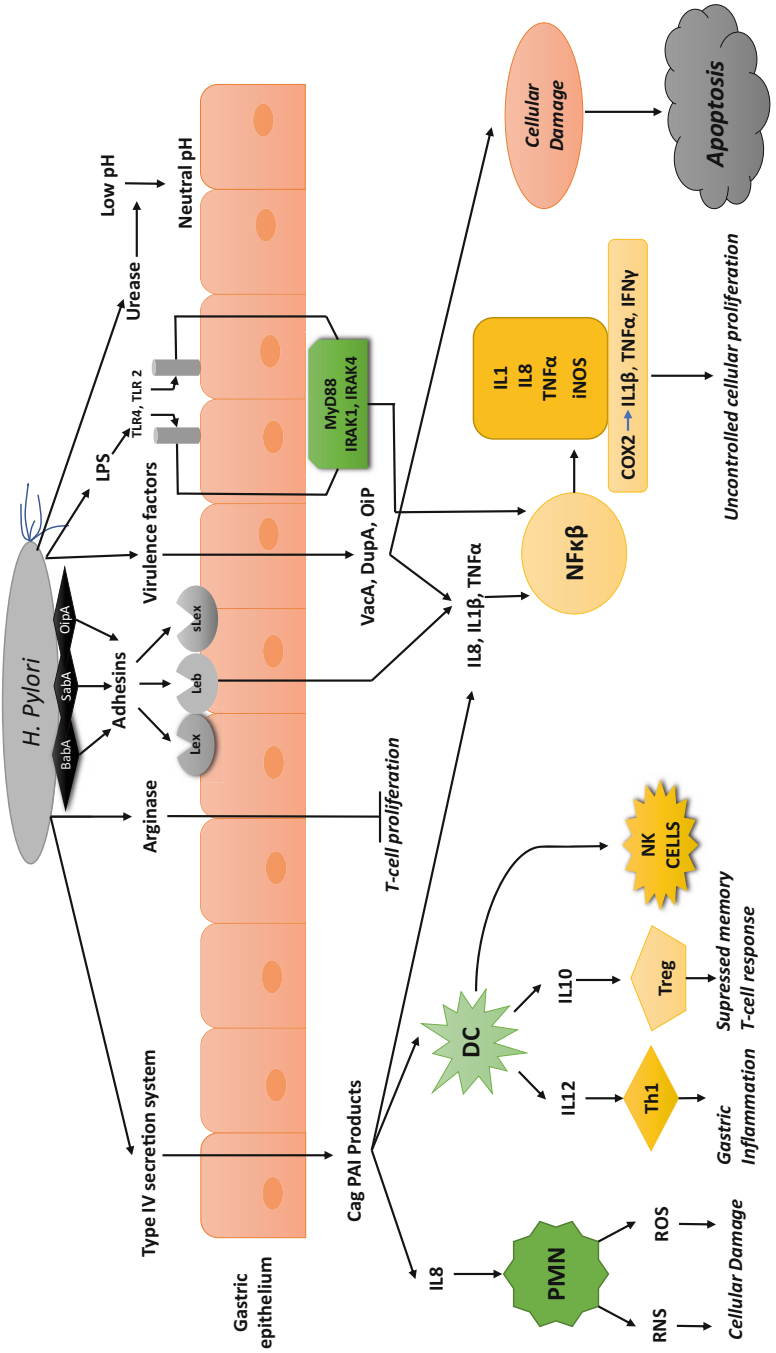

Fig. 9.1 Pathogenesis of *H. pylori* infection and its various outcomes

with a PPI and amoxicillin is combined with and followed by triple therapy with a PPI, clarithromycin, and tinidazole or metronidazole for another 5 days. Although the success rate of this sequential therapy is much better than conventional 5 days or 10 days triple therapy, several side effects are also observed at the end of the treatment (Nyssen et al. 2016; Fallone et al. 2016).

Due to well-known side effects of antibiotic consumption and emergence of resistant bacteria, a new alternative therapeutic and preventive agent of microbial origin could be potential candidate to counter acute infections at an early stage. Some lactic acid bacteria (LAB) of human origin including genus *lactobacilli* and *Bifidobacterium* have shown anti-*H. pylori* activities and therefore could serve as potential source of antimicrobial agent by displacement as well as inhibition of pathogenic *H. pylori.* In this context, probiotics have recently emerged as safe, cost effective, and easily affordable as prophylactics or biotherapeutics for general health promotion and specifically in the management of the target disease particularly *H. pylori* related inflammatory diseases. Probiotic microorganisms may induce its effects by inhibiting *H. pylori* growth, reducing on-going inflammation, and competing against it (Ayala et al. 2014).

9.2 Probiotic Microorganisms

Probiotics are defined as "live microorganisms which when administered in adequate amount provide health benefits in host" (Hill et al. 2014). Microorganisms such as lactic acid-producing bacteria (*Lactobacillus* spp. and *Bifidobacterium* spp., *Bacillus* spp.) and other species of bacteria (*Escherichia coli*, *Saccharomyces boulardii*, and *Streptococcus thermophilus)* show probiotic attributes (Ahire et al. 2011; Ahire et al. 2013; Ahire et al. 2010). Probiotics show a diverse array of biological effects after their consumption. Probiotic strains are capable of synthesizing antimicrobial compounds such as organic fatty acids, ammonia, hydrogen peroxide, bacteriocins, and biosurfactant (Arqués et al. 2015; Rushdy and Gomaa 2012). Their presence can readily diminish the amount of nutrients and spaces required for pathogenic growth and adherence, respectively. Probiotics can occupy a large number of receptor sites. After binding with specific carbohydrate receptors which are otherwise available for pathogen binding, probiotics can therefore reduce pathogen adhesion to epithelium cells (Monteagudo-Mera et al. 2019; Lee and Puong 2002). Immune system can also be modulated by probiotics mainly through synthesis of pro- and anti-inflammatory cytokines, stimulation of host dendritic cells (DCs), modulation of nuclear factor-κB (NF- κB) pathway, etc. (Azad et al. 2018; Plaza-Diaz et al. 2019; Llewellyn and Foey 2017). Several in vivo and in vitro studies have reported the beneficial effects of probiotics against *H. pylori* infection and based on these reports a new therapeutic approach can be designed to eradicate *H. pylori* associated diseases (Del Giudice et al. 2009; Sommi et al. 1996). In various gastrointestinal diseases including antibiotic-associate diarrhoea, probiotics have shown beneficial effects and it has been used widely (FAO/WHO 2001; Behnsen et al. 2013; Petschow et al. 2013; Sarowska et al. 2013; Foligné et al. 2013). Several studies were performed and

demonstrated positive effects of probiotics on bacterial inhibition and prevention (Guarner et al. 2011). Three meta-analysis were conducted on the effects of probiotic supplementation in *H. pylori* eradication therapy and it was concluded that probiotics exerted beneficial effects on eradication treatment, increasing eradication rates significantly (Hamilton-Miller 2003; Wang et al. 2013). For the treatment of peptic ulcer disease induced by *H. pylori*, vaccination could represent a potent alternative approach as antibiotic treatment comes with too many problems. Although a large number of pre-clinical studies with vaccine candidates were published, however, a few were used in clinical studies and they also failed to show any efficacy against *H. pylori* (Zheng et al. 2013). In the present situation, therefore, probiotic treatment could create a new era of microbial interference therapy without any adverse effect.

9.3 Mode of Action of Probiotics

Probiotic strains have the capability to induce mucosal immune system by stimulating the activation of resident macrophages thus increasing antigen presentation and modulating cytokine profile. For example, *H. pylori* infected children when supplemented with probiotics-containing yogurt, a higher level of serum IgA, and reduced serum interleukin 6 (IL-6) were observed (Li et al. 2014). In addition, probiotics may also exert its effects through non-immune mechanisms such as antagonistic and/or competitive effects against pathogenic organisms. Probiotics are also capable of producing antioxidants and antimicrobial substances, can enhance intestinal barrier function by modulating tight junctions between epithelium, stimulates mucin production, can maintain local pH balance and many other undefined functions (Del Giudice et al. 2009; Yang and Sheu 2012).

9.4 Substances Produced by Probiotics that Inhibit or Kill *H. pylori*

A variety of substances are produced by probiotic microorganisms that either inhibit *H pylori* or induce an antibody response but the exact mechanism is not clearly understood. Anti-bacterial compounds such as bacteriocins, lactic acid, acetic acid, and hydrogen peroxide (H_2O_2), etc. are generally produced by various strains of probiotics (Table 9.2). During initial periods of infection, *H. pylori* uses urease enzyme to neutralize local acidic environment for their survival. *Lactobacillus casei* (*L. casei)* has shown to inhibit the action of urease by secreting lactic acid (Guarner et al. 2011; Ljungh and Wadström 2006). Catalase activity is another characteristic of *H. Pylori* and many probiotic strains are capable of producing H_2O_2. This catalase activity induces the production of many free radicles which act as an anti-bacterial agent. In vitro as well as in vivo studies have shown that culture supernatant of *L. johnsonii* La1 and *L. acidophilus* LB can inhibit *H. pylori* effectively (Aiba et al. 1998; Sgouras et al. 2004). In in vitro studies, *H. pylori* can be killed with heat-

Table 9.2 Various antimicrobial substances released by probiotic strains

Probiotic microorganism	Antimicrobial substances
L. acidophilus 4356	Bacteriocin
*L. salivarius*WB1040	Salivaricin B
L. casei Shirota	Bacteriocin (heat-labile substance)
L. acidophilus LB	Heat-stable protein
L. lactis BH5	Bacteriocin
L. Acidophilus	CRL639 autolysins
W. confusa PL9001	Acteriocin
*L. johnsonii*La1	Heat-stable substance
L. Acidophilus	Bacteriocin
L. reuteri TM 105	Glycolipid-binding proteins
B. subtilis 3	Anticoumacin A, B, C
*L. reuteri*ATCC 55,730	Reuterin

inactivated *L. johnsonii* No. 1088 (HK-LJ88) and when HK-LJ88 and *H. pylori* are co-cultured, *H. pylori* shows changed morphology and lysis. In another study, *H. pylori* is co-cultured with *L. acidophilus* CRL 639. After 24 h, *L. acidophilus* was found to be lysed completely and proteins released from it killed *H. pylori* (Michetti et al. 1999). However, the exact nature and the mechanism of action of these antimicrobial compounds are yet to be discovered. Probiotic strains can produce bacteriocin which is considered as one of the important properties (Michetti et al. 1998). For example, *Bacillus subtilis* can inhibit *H. pylori by* the production of animocumacins, an isocoumarin antibiotic (Lorca et al. 2001; Dobson et al. 2012). *L. Reuteri* ATCC 55730 secretes reuterin which is reported to inhibit VacA gene expression of *H. pylori* (Pinchuk et al. 2001).

9.5 Competition for Colonization

Adhesion of *H. pylori* in the gastric epithelium is required in the first place for colonization and initiates infection. *H. pylori* is equipped with various adhesions and outer membrane proteins such as neutrophil activator protein, fibrillar N-acetylneuraminyl lactose-binding hemagglutinin (NLBH), Bab A, Lewis antigen, heat shock protein, Alp A, and Alp B which facilitates its binding to mucin receptors, mucopolysaccharide receptors, Lewis blood group substances, glycolipid receptors, and other corresponding receptors present in the gastric epithelium. Probiotics can inhibit this binding process by various mechanisms. Probiotics can compete with *H. pylori* for adhesion sites and nutrients, can cause steric hindrance for available space and by secreting antimicrobial compounds they can reduce the number of *H. pylori* attached to the epithelium. Several studies have shown that probiotics can actually bind to binding receptor and reduce *H. pylori* adhesion. *L. reuteri* can compete with *H. pylori* for specific binding receptors such as asialo-GMI and sulfatide and reduces *H. pylori* adhesion (Nam et al. 2002). In vitro studies with potential probiotics strains such as *L. acidophilus* LB, *L. salivarius, L. johnsonii, and*

W. confuse strain PL9001 have shown that these strains exclude *H. pylori* by competition (Michetti et al. 1998, 1999; Urrutia-Baca et al. 2018; Mukai et al. 2002). Other studies have revealed that *lactobacillus* growth is antagonistic for *H. pylori* and a higher level of *lactobacillus* in stomach reduces the amount of *H. pylori* (Kabir et al. 1997). *Lactobacillus* can also downregulate *H. pylori* adhesin molecule sabA and reduces its attachment to gastric mucosa (Nam et al. 2002). Although several other studies like mentioned above have provided numerous insights still a lot has not been discovered yet and it would be interesting to investigate further about potential molecular mechanism of probiotics and their interaction with binding receptors.

9.6 Effects on Mucosal Barrier

There are three main barrier systems which is present in normal stomach to maintain the epithelial integrity:

9.6.1 The Pre-Epithelial Barrier

This barrier is made up of a mucus-bicarbonate-phospholipid layer and it is located between the gastric lumen and the epithelium**.**

9.6.2 The Epithelial Barrier

This barrier consists of epithelial cells connected by tight junctions and secretes trefoil factors, prostaglandins, and heat shock proteins**.**

9.6.3 The Subepithelial Barrier

This barrier is composed of mainly capillaries and sensory innervations. Peptic ulcer and/or gastric ulcer may develop if one of these barrier systems gets damaged. Probiotics have the capacity to up-regulate prostaglandin and mucous secretion, tight junction protein expression and cell proliferation, and inhibition of apoptosis (García et al. 2012; de Klerk et al. 2016; Uchida and Kurakazu 2004; Lam et al. 2007; Madsen et al. 2001) (Fig. 9.2). With the help of virulence factors such as CagA and VacA, *H. pylori* damages gastric mucosa and inhibits mucous secretion (Gotteland et al. 2001b; Rao and Samak 2013). In addition, *H. pylori* can supress MUCI and MUC5A gene expression and reduce epithelium stability by destroying mucosal barrier (Backert et al. 2016). In studies it was shown that probiotics induce mucous secretion and when colonic epithelial Caco-2 cells or colorectal HT29 cells are treated with probiotics, production of mucin is increased (Lesbros-Pantoflickova et al. 2007; Lesbros-Pantoflickova et al. 2000; Mattar et al. 2002). Other studies have

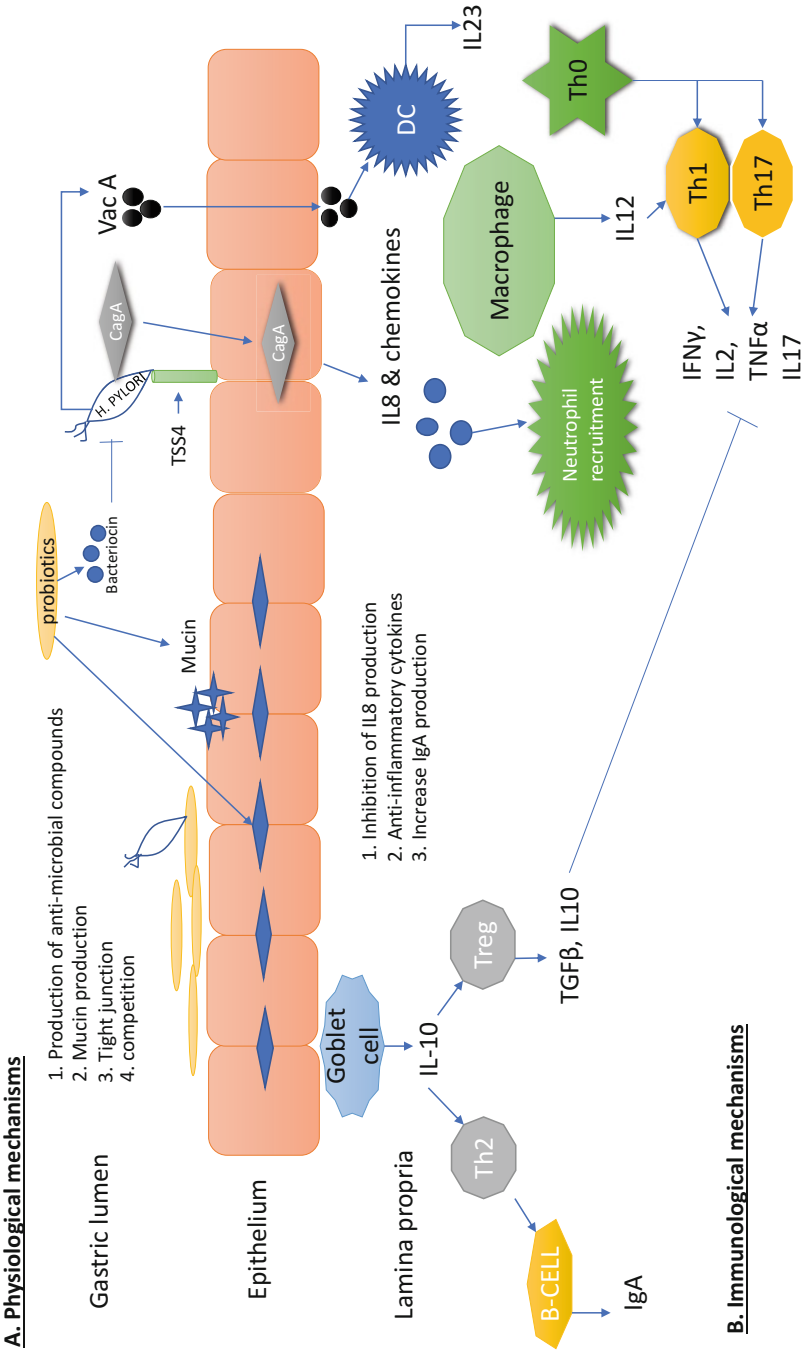

Fig. 9.2 Various mechanisms by which probiotics may exert their effects

also shown that when VSL#3, a probiotic mixture, was administered for seven days to rats, a 60-fold increase in MUC2expression and secretion was observed (Kim et al. 2008). The same probiotic mixture, VSL#3, can up-regulate the expression of tight junction proteins (occludin and zonula occludens-1) via the activation of p38 or mitogen-activated protein (MAP) kinase and extracellular signal-regulated kinase (ERK) signalling pathways (Otte and Podolsky 2004).

9.7 Influence on Immunity and Inflammation

Initially the main characteristic of *H. pylori* infection is gastric mucosal inflammation and it begins with Correa cascade, from chronic gastritis to atrophic gastritis to intestinal metaplasia to atypical hyperplasia, culminating in gastric cancer. Due to *H. pylori* infection inflammatory mediators such as various cytokines and chemokines are also released. Urease, cytotoxin-associated geneA (CagA), vacuolating cytotoxin A (VacA), and neutrophil activating protein (NAP) are major virulence factors of *H. pylori.* NAP activates neutrophils and promotes the production of reactive oxygen species in neutrophils (Fig. 9.1). Cytokine IL-8 is released initially and it leads to migration of more neutrophils and monocytes to the mucosa (Caballero-Franco et al. 2007). This leads to the activation of monocytes, dendritic cells, and CD4+ T cells (type 1) in the lamina propria which produces TNF-α as well as IL-1, IL-6, IL-4, IL-5, and interferon-γ (INF-γ) cytokines (Dai et al. 2012; Arakawa et al. 1997; Noach et al. 1994). This continuous response induces inflammation. It has been reported that probiotic strains such as *L. bulgaricus, L. acidophilus, and L. salivarius* can downregulate IL-8 secretion by *H. pylori* (Michetti et al. 1999; Cassatella et al. 1993; Harris and Smith 1997). Various animal investigations had revealed the several beneficial effects of probiotics on immune system although probiotic induced immune responses depend on the host's immune status. These immune modifying mechanisms of probiotics may be mediated by interacting with epithelial cells binding to the TLRs that are expressed on the surface of the epithelial cells and initiate immune defence mechanism and controlling the cytokines balance and mainly inflammatory/anti-inflammatory chemokines which ultimately reduces gastric activity and inflammation (Blum et al. 2002) (Fig. 9.2). In several animal and in vitro studies it was shown that following probiotic intake such as *L. salivarius,* the level of *H. pylori*-stimulated secretion of IL-8 by gastric epithelial cells was inhibited and decrease in specific IgG antibodies to *H. pylori* infection was observed. It results a diminished level of gastric inflammation (Michetti et al. 1999; Gill 2013). Due to *H. pylori* infection a higher level of Smad7 and NF-kB is produced and induces inflammation and recent research by (Yang et al. 2012) has shown that pre-treatment with *Lactobacillus acidophilus* at higher doses could reduce inflammation through inhibiting *H. pylori*-induced transduction of Smad7 by the inactivation of Jak1 and Stat1 pathways and followed by decreased production of NF-kB. Finally, probiotic intake has been shown to stimulate IgA production, resulting in a strong mucosal barrier (Yang et al. 2012). A probiotic mixture of *Enterococcus faecalis*, *B. longum*, and

L. acidophilus could reduce the release of inflammatory factors such as TNFα, IL-1β, IL-10, IL-6, granulocyte colony-stimulating factor, and macrophage inflammatory protein 2 by inhibiting the NF-κβ and mitogen-activated protein kinase signal transduction pathways. In experimental mice *L. casei* strain Shirota decreased *H. pylori*-mediated inflammatory responses. Thus, it is evident from various animal studies that probiotics are significantly effective to reduce the degree of inflammation and outcome of *H. pylori* infection. The effect of probiotics on the immune responses is difficult to generalize not only because it is highly dependent on host's immune status but also different probiotics strains generate divergent immune responses.

9.8 Future Perspective

In an attempt to manage the *H. pylori* infection, it is very crucial to recognize in depth of interface between *H. pylori* and probiotics. The eradication of *H. pylori* by conventional triple therapy using double antibiotics and proton pump inhibitors is relatively low and ranges from 70% to 80%. The conventional therapy may produce antibiotics resistant strains and several complications in patients. The antibiotic associated gastrointestinal complications are main cause for lower conformity. Therefore, alternative therapeutic and preventive agents of microbial origin could be potential mediator to counter acute infections at an early stage. Probiotics like several *Lactobacillus* spp., *Bifidobacterium* spp., and *Lactococcus lactis* have been investigated as a therapeutic supplement to reduce the side effects of conventional antibiotic-based *H. pylori* treatments. Probiotic *lactobacilli* of human origin including L. *casei* Shirota, *L. johnsonii* La1, and *L. rhamnosus* GG have shown anti-*H. pylori* activities and therefore could serve as potential source of antimicrobial agent by displacement of *H. pylori*. Probiotics microorganisms have various mechanisms to inhibit pathogen proliferation, competitive displacement, immunomodulation, and to maintain mucosal integrity, etc. Some studies suggest that prebiotics and some potential probiotics bacteria show promising results as an adjuvant treatment in reducing side effects. The balance diet and right nutrient factors are also important criteria to manage and reduce *H. pylori* infection and associated problems. Administration of functional food contacting probiotic to children in from early childhood can reduce the incidence of *H. pylori* colonization in the general population. The highly awakened new generation is now looking for safe natural products as an alternative to the conventional drug-based therapy for routine health care and disease management. Therefore, probiotics have recently emerged as safe, cost effective, and easily affordable as biotherapeutics for general health promotion and specifically in the management of *H. pylori* associated disease. Further, investigation is required to work out better combinational approach containing probiotic and conventional drug for the treatment of *H. pylori* infections to avoid any side effect.

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Designing Probiotics and Its Clinical Applications

10

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Abstract

Probiotics are health beneficial microorganisms which may improve the disturbed gut microflora and gut disease condition. Various probiotic preparations are available as health supplements in the market. Due to advancements in modern recombinant DNA technology and molecular biology techniques, alteration of DNA is possible. By using the above-mentioned techniques, probiotics can be improved in a number of ways, such as robustness and stress tolerance. Designer probiotics are ought to target enteric infections via obstruction of host-pathogenic ligand–receptor interactions. The probiotic's interaction with the host cell receptors can be used as a tool for targeting infections. Recently, their clinical applications have been exploited in various therapeutic areas such as vaccines. Various microbial genera and their species used as probiotics are *Lactobacillus, Bifidobacterium, Escherichia, Bacillus,* and *Streptococcus.* Probiotics have shown clinical applications in *Helicobacter pylori* infections, inflammatory bowel disease, antibiotic associated diarrhea, ulcerative colitis, and allergy. There are various applications of designer probiotics, such as in the treatment of enteric infections, inflammatory bowel disease, diarrhea, dysentery, etc. Designer probiotics are emerging in the area of treatment of therapeutic diseases. In this chapter, various clinical applications of these probiotics are discussed, such as immune related diseases, enteric infections, HIV infection, microbial infections, cancer, etc.

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10.1 Introduction

Probiotics are the beneficial bacteria used as food supplement. Not all bacteria are harmful to humans, some are useful also. Certain bacteria such as *Lactobacillus casei* can help in digestion, to improve condition of gut affected due to certain pathogens. Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) (2002) defines Probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (Hill et al. 2014). According to WHO guidelines for probiotics, the phenotype as well as genotype-based strain identification is must for probiotic for food use. They should go through safety assessment for food use and have proof of a healthful effect. Clinical trials should carry out for food use. Probiotics can be used as therapeutic agents for gastrointestinal related diseases such as inflammatory bowel disease (IBD) (Coqueiro et al. 2019), irritable bowel syndrome (IBS) (Dale et al. 2019), antibiotic associated diarrhea (Mekonnen et al. 2020; Cremonini et al. 2002), ulcerative colitis (Bjarnason and Sission 2019), allergy (Hajavi et al. 2019; Wang et al. 2019), *Helicobacter pylori* infections (Qureshi et al. 2019), *Salmonella* infections (Rokana et al. 2017), Shiga toxigenic *Escherichia coli* (STEC) infections (Giordano et al. 2019), enteropathogenic *Escherichia coli* (EPEC) infections (Walsham et al. 2016), enterohemorrhagic *Escherichia coli* (EHEC) infections (Cordonnier et al. 2017), and uropathogenic *Escherichia coli* (UPEC) infections (de Llano et al. 2017).

Recombinant DNA technology and molecular biology techniques are useful for designing of the probiotics. Numerous microbes have the potential to use as probiotics (Fijan 2014). It is necessary that they should be non-infectious otherwise difficult to use. Desired probiotic microorganism candidate should satisfy "generally recognized as safe" (GRAS) guidelines (Hoffmann et al. 2014). A number of in vivo and in vitro studies such as gastric acid resistance, bile resistance, mucosal and human epithelial cells adhesion study, antimicrobial activity versus pathogenic bacteria and activity of bile salt hydrolase are used for the screening of probiotic candidate (Papadimitriou et al. 2015). Their survival through the extreme gut conditions (bile acid, pH, osmolarity, etc.) are the limiting conditions in effective designing. During their formulation and transportation some other external factors also contribute to the stress environment (Fiocco et al. 2020). Thus, a sturdy design is required to withstand stress environment.

They can exert their effect by producing lactic acid, hydrogen peroxide (H_2O_2), bacteriocins, antiviral compounds, short chain fatty acids (SCFA), antimicrobial

peptides and proteins (AMPs). These metabolic products are responsible for destruction of harmful microbes responsible for diseases (Kaur et al. 2002). Therapeutic applications and mechanism of action of probiotics are shown in Fig. 10.1. Various probiotics are marketed nowadays as a health supplementary product but not as therapeutics because of clinical trials. Various publications reported that they can be used as receptor mimicking strategy to remove harmful viruses and bacterial adhesion with gastrointestinal tract cells (Paton et al. 2006, 2010b).

The classification of probiotics based on colonization in intestine is explained in Table 10.1. Resident strains are generally available in human gut microbiota. Resident microorganisms available in probiotic preparation can restore in gut microbiota. Resident strains prevent the colonization of transient strains in gut. Transient strains ephemerally assimilate in gut microbiota. They can be used as delivery vehicle to transport protein vaccine. Transient strains cannot restore themselves in the gut (Amalaradjou and Bhunia 2012).

There are various clinical applications of probiotics (Steidler 2003). List of the probiotics and their uses is given in Table 10.2. There are two generations of probiotics. Traditional or First-generation probiotics (bacterial therapeutics)—Natural strains without tampering their genes used for prevention and treatment of diseases. These are general probiotics mainly from *Lactobacillus* and *Bifidobacterium* genus. Various species of *Lactobacilli* have therapeutic effect in case of enteric infections.

10.1.1 Next or Second-Generation Probiotics (Bacterial Therapeutics)

Genetically engineered or tailored strains. These probiotics can be made more robust and stronger enough to survive in the gut. These probiotics also called designer probiotics. They can be used as therapeutics in case of inflammatory bowel disease (Sireswar et al. 2019; Martín and Langella 2019).

Pharmabiotic is a term which consists of therapeutic use of genetically engineered commensal bacteria, prebiotics, synbiotics, probiotic bacteria, their metabolites, viable and nonviable microorganisms with pharmacological effect in health or disease (O'Hara and Shanahan 2006, 2007). One group reported the effect of probiotics on experimental colitis in mice. In this study the better effect on colitis condition was exerted by DNA of probiotics rather than probiotics metabolites and colonizing ability. Nonviable probiotics are equally effective as viable probiotics (Rachmilewitz et al. 2004).

10.1.2 Disadvantages of Traditional Probiotics

There are many drawbacks or limitations of traditional probiotics such as lack of specificity as they have wide spectrum of antibacterial activity. Some of the probiotics have lesser efficacy such as *Listeria monocytogenes*, due to its inability

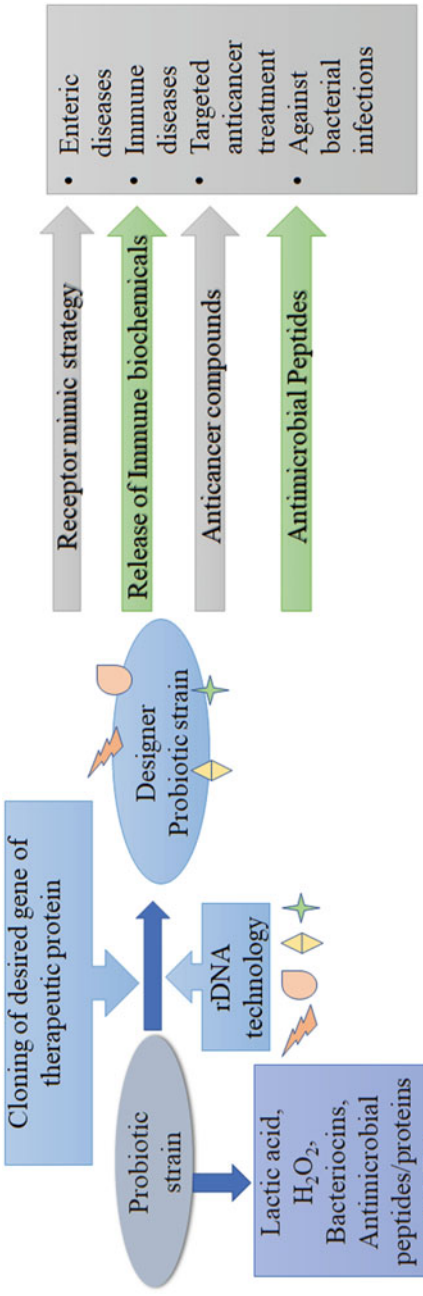

Fig. 10.1 Schematic representation of mechanism of action and therapeutic application of designer probiotics

Table 10.1 Probiotics classification on the basis of colonization in intestine (Amalaradjou and Bhunia 2012)

Resident strain	Transient strain
Lactobacillus acidophilus	*Lactobacillus casei*
Lactobacillus salivarius	*Lactobacillus rhamnosus GG*
Bifidobacterium bifidum	*Lactobacillus bulgaricus*
Bifidobacterium infantis	*Lactobacillus yoghurti*
Bifidobacterium longum	*Lactobacillus brevis*
Bifidobacterium animalis	*Lactobacillus kefir*
Streptococcus faecalis	*Lactobacillus delbrueckii*
Streptococcus faecium	*Lactobacillus plantarum*
	Streptococcus lactis
	Streptococcus thermococcus

Table 10.2 List of probiotics and their use

Sr. No.	Probiotics	Model	Clinical applications	References
1	*Escherichia coli* Nissle 1917	Human	Ulcerative colitis	Rembacken et al. (1999), Kruis et al. (1997)
2	*VSL#3 - Bifidobacterium, lactobacillus, and Streptococcus*	Human	Ulcerative colitis Chronic pouchitis	Venturi et al. (1999) Gionchetti et al. (2000)
3	*Lactobacillus plantarum*	Human	Irritable bowel syndrome	Nobaek et al. (2000)
4	*Lactobacillus rhamnosus GG*	Human	Crohn's disease, Atopic eczema, dermatitis, allergy (cow's milk)	Gupta et al. (2000) Majamaa and Isolauri (1997)
5	*Bifidobacterium bifidum*	Human	Acute diarrhea and Rota virus	Saavedra et al. (1994)
6	*Streptococcus thermophilus*			
7	*Saccharomyces boulardii*	Human	Crohn's disease, diarrhea	Guslandi et al. (2000)
8	*Lactobacillus fermentum* NCIMB 5221 and 8829	Invitro-Caco-2 human epithelial colorectal cancer adenocarcinoma cell	Colorectal cancer	Kahouli et al. (2015)
9	*Lactobacillus gasseri* BNR17	Human	Anti-obesity	Kim et al. (2018)

to obstacle the adhesion and colony formation (Karimi and Peña 2008; Koo et al. 2012; Mathipa and Thantsha 2017). Due to the above limitations, it is necessary to improve the effectiveness of probiotics against various infections, stress tolerance, etc. To overcome these problems, designing of probiotics has come into the picture. The mechanism of action natural and designer probiotics is shown in Fig. 10.1.

10.2 Designer Probiotics in Enteric Diseases and Disorders

Receptors are the structures which are responsible for the binding of various molecules to exert changes in the cells and/or to initiate certain chain reaction to produce chemical mediators. In gastrointestinal tract infection, the toxin produced from pathogen can bind to the receptor (oligosaccharides) present on glycoproteins on the host cells and invade into the cell and disturb the requisite functions and cycles. In the treatment of various gastrointestinal tract infections, the main mechanism is to prevent toxin attachment to oligosaccharide receptors displayed on glycoproteins on host cells. The mechanism of designer probiotics as a receptor mimic strategy is explained in Fig. 10.2.

In the case of cholera infection, *Vibrio cholerae* secretes cholera toxin (Ctx), from AB_5 toxin family containing A subunit and B subunit (pentamer), the former is responsible for delivering the machinery inside the cell to take over and disrupts the cell function and the latter is necessary for binding to the receptor (Beddoe et al. 2010). Heat labile enterotoxin produced by enterotoxigenic *Escherichia coli* (ETEC) is also from AB_5 toxin family (Duan et al. 2019). Shiga toxigenic *Escherichia coli* (STEC) secretes Shiga toxin (AB_5 toxin family) (Krause et al. 2018). One group developed toxin binding probiotic by expressing genes of glycosyltransferase from *Neisseria gonorrhoeae* and *Campylobacter jejuni* in anavirulent *Escherichia coli*, resulting into a chimeric lipopolysaccharide. This recombinant microbe has the ability to bind cholera toxin with better avidity and able to adsorb significant amount of purified cholera toxin (Focareta et al. 2006).

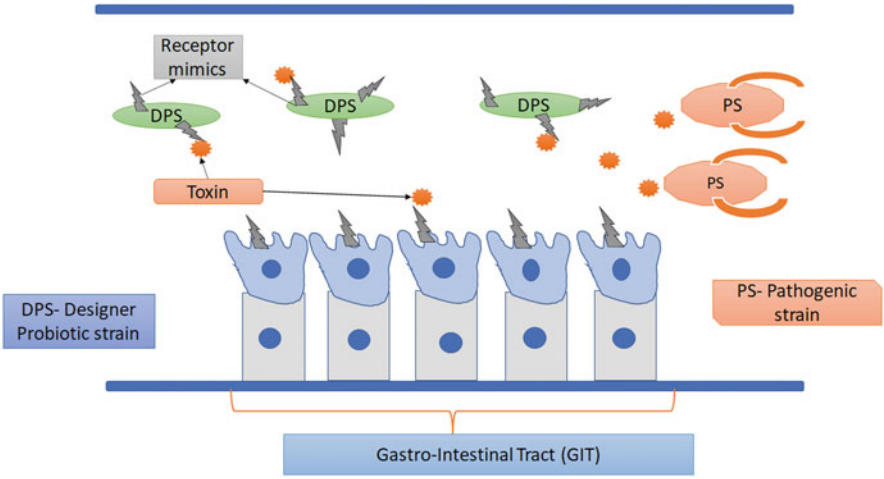

Fig. 10.2 Designer probiotics as a receptor mimic strategy for the prevention of enteric infection caused by pathogenic strain producing toxin. (The figure depicts the protection of gastrointestinal tract cells from the toxin secreted by pathogenic strain. Toxin will bind to the receptors expressed by probiotic strain which was previously engineered with the gene responsible for host receptor mimics)

Paton et al developed a new strategy for probiotics against enteric infection by expressing mimics of host's oligosaccharide receptor on the non-virulent bacterium surface which is capable to survive in gut. This was carried by manipulating the exterior core region of the lipopolysaccharides with the help of cloning and expression of heterologous glycosyltransferase gene. The chimeric lipopolysaccharide molecule was added in the exterior membrane and displayed as planar arrangement (Paton et al. 2010a, b).

Paton et al demonstrated the study for the prevention of Shigatoxigenic *Escherichia coli* infection through genetic engineering of *Escherichia coli.* Shigatoxigenic *Escherichia coli* (STEC) strain *Shigella dysenteriae* type 1 secretes shiga toxin (Stx), which is responsible for adhesion and invasion in gastrointestinal tract cells and infection. Infection results in hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). They had isolated genes of galactosyltransferase from *Neisseria meningitidis* and *Neisseria gonorrhoeae* and cloned into *Escherichia coli* (non-virulent). Toxins bind to *Escherichia coli* expressing receptors, which mimic receptors of the host cells. The results showed that recombinant *Escherichia coli* was found to be highly potent against shiga toxigenic *Escherichia coli* infection bearing mice (Paton et al. 2000).

Paton et al also showed the effect of recombinant probiotics on the enterotoxic *Escherichia coli* produced heat labile enterotoxin induced diarrhea. They had used nonpathogenic strain of *Escherichia coli* CWG308. Expression of glycosyltransferase gene was done in *Escherichia coli* CWG308. These genes were obtained from *Neisseria meningitidis* or *Campylobacter jejuni*. This recombinant *Escherichia coli is* capable to secrete chimeric lipopolysaccharide, which is responsible for the attachment of heat labile enterotoxin to it. The robust binding of toxin to receptor was observed in *Escherichia coli* expressed lacto-N-neotetraose. It counteracts more than 93% activity of heat labile enterotoxin in cell lysate of enterotoxigenic *Escherichia coli* (ETEC) strains originated from human as well as swine. The other *Escherichia coli* with ganglioside GM2 construct showed lesser neutralization against toxin. Though this type of approach has some disadvantages such as toxin can neutralize but it would not affect the pathogenic strain population in terms of survival and replication (Paton et al. 2005).

Hostetter et al. studied the effect of receptor mimic probiotics on Shiga toxigenic *Escherichia coli* (STEC) infected porcine for prevention of HUS therapy. Recombinant *Escherichia coli* (receptor mimic probiotic) were orally administered to the pigs infected with shigatoxigenic *Escherichia coli* (STEC) strain. The animal study results showed the significant decrease in Stx in the gut . But, It failed to decrease the systemic adsorption of toxin. (Hostetter et al. 2014).

10.3 Designer Probiotics in Immunity Related Diseases and Disorders

Probiotics are beneficial in inflammatory and autoimmune diseases. One of the edges of probiotics is in immune system regulation. In case of immunodeficiency, probiotic strains stimulate the immune response. In the case of rheumatoid arthritis which is the overstimulation of immune response, probiotics may suppress immune response. Immunity related diseases like rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease (IBD), ulcerative colitis, Crohn's disease, multiple sclerosis can be cured by using probiotics. Various metabolites are produced by probiotics such as short chain fatty acid (SCFA), dietary tryptophan and indole derivatives, which are having anti-inflammatory effect (Liu et al. 2018).

Braat et al. reported one study related to Crohn's disease treated with genetically engineered *Lactobacillus lactis.* It was done by eliminating gene responsible for thymidylate synthase activity and replaced it with human Interleukin-10 (IL-10) gene. This results showed the decrease in disease state (Braat et al. 2006).

Vandenbroucke et al genetically engineered *Lactococcus lactis* for the production of tumor necrosis factor (TNF) neutralizing antibody fragments as a therapy for inflammatory bowel disease (IBD). The therapeutic effect of bivalent antibody vs dextran sulfate sodium (DSS) induced chronic colitis was studied on murine models. These antibody fragments showed remarkable decrease in inflammation of DSS induced chronic colitis mice (Vandenbroucke et al. 2010).

For the treatment of inflammatory diseases, genetically engineered *Lactobacilli* species had been tailored to perform delivery of therapeutic proteins in gastrointestinal tract (Maddaloni et al. 2015). They reported that the feeding of *Lactococcus lactis* overexpressed with colonization factor antigen I (CFA/I) to the arthritic mice in the form of fermented milk. The results demonstrated that arthritis relevantly improved by the way of CD39+ regulatory T cells (T_{regs}), which produces TGF-β and IL-10 responsible for effective suppression of TNF-α production and entry of neutrophils into the affected part of joints. They also promoted the new concept of bacterial fimbriae, albeit it is virulence factor, it can be used as therapeutic tool to alter host immune system to prevent inflammation (Chua et al. 2017; Maddaloni et al. 2015).

10.4 Designer Probiotics with Antimicrobial Peptides

Due to the heavy use of antibiotics several microbes become resistant to them. Hence, novel therapy is necessary to overcome this problem. Various strains are becoming multidrug resistant. Probiotics are also the bacteria but with beneficial effect to human gut and microbiota (Mathipa and Thantsha 2017). Antimicrobial peptides (AMPs) are secreted by probiotics as a defence mechanism. There are several reports available about the use of antimicrobial peptides against multidrug resistant pathogens (Mandal et al. 2014).

Traditional methods of production of antimicrobial peptides are costlier and tedious process. The common problem for the delivery of the peptides is the degradation while administered through mouth and through intravenous route by immune system. Hence, there is the need to develop a new system to deliver antimicrobial peptides. Probiotics can be used as dual strategy as they can produce antimicrobial peptides at the desired site as well as give beneficial effect to the gastrointestinal tract. *Lactobacilli* are the main strains for the use of recombinant technology. They are robust and can tolerate stresses from gastrointestinal tract environment and prone to the expression of genes for production of antimicrobial peptides. Various studies suggested that the use of bioengineered *Lactococcus lactis* containing antimicrobial peptides producing genes has significant inhibition of *Escherichia coli* and *Salmonella* (Mandal et al. 2014).

Volzing et al carried out studies regarding the production of antimicrobial peptides Alyteserin-1a and A3APO from *Lactococcus lactis* (Volzing et al. 2013). Both peptides have sufficient activity for the inhibition of pathogenic *Escherichia coli* and *Salmonella*. They had screened various antimicrobial peptide candidates. These peptides were synthesized by solid state synthesis and subjected against indicator *Escherichia coli* and *Salmonella* strains. Amongst them A3APO and Alyteserin gave best results for high antimicrobial activity on *Escherichia coli* and low activity against host cells, i.e., *Lactococcus lactis.* For the construction of recombinant *Lactococcus lactis* strain, they had synthesized codon optimized nucleotide sequences of two peptides and fused to USP45 single peptide sequence. Transformation of the *Lactococcus lactis* was done by using a nisin inducible promoter nis-A controlled cloning of expression cassette. Recombinant strains were induced with nisin A for the production of both peptides. Effective results were obtained after the testing of supernatant containing both peptides against pathogenic *Escherichia coli* and *Salmonella*. They successfully inhibited both pathogenic strains but maintain the viability of *Lactococcus lactis.* Hence, these peptide gene containing lactic acid bacteria may be used as targeted antimicrobial therapy against Gram negative pathogenic bacteria and for the production as well as delivery (Volzing et al. 2013).

Antimicrobial peptides have direct action on the microorganisms as well as immunity of host by elicitation of modulation of cytokines and chemokines, programmed cell death, immune cells modulation as well as differentiation of adaptive immunity (Mahlapuu et al. 2016; Mandal et al. 2014).

A number of antimicrobial peptides are cloned and expressed in heterologous system of probiotics (Mandal et al. 2014). *Lactobacillus lactis* is the versatile bacteria, which can be used as host to express certain genes such as ABP-118 (Flynn et al. 2002), lactococcin A (Chikindas et al. 1995), hiracin JM79 (Sánchez et al. 2008), Enterocin P (Gutiérrez et al. 2006), Alytesrin and A3APO (Volzing et al. 2013). *Lactobacillus salivarius* was used for the production of bactofecin A (O'Shea et al. 2013). *Lactobacillus sakei* was used for the production of pediocin PA-1, sakacin P, entercin A, leucocin A and curvacin A (Johnsen et al. 2005). *Lactobacillus plantarum and Bacillus cereus* were used for the production of ABP-118 (Flynn et al. 2002).

Bacteriocins are important type of peptides produced by the various microbes. Bacteriocins are the distinguished characteristic of probiotics (Dobson et al. 2012). Bacteriocin production has the main importance while selecting probiotic strain. Several studies illustrated the effect of bacteriocin production on the potential of a strain to compete with others in the gastrointestinal tract (Corr et al. 2007). Bacteriocins are peptides produced by bacteria, which can act against other bacteria (Cotter et al. 2005; Kullen and Klaenhammer 2000). They consist of a heterogenous group of peptides in respect of physical parameters, immune response, mechanism of action, target cell receptors, antimicrobial potency, etc. Bacteriocins could support to probiotic function in a number of ways. Van Zyl et al studied the effect of plantaricin 423 and mundticin ST4SA bacteroicins against *Listeria monocytogenes* EGDe in the murine models. Plantaricin 423 was secreted from *Lactobacillus plantarum* 423 and mundticin ST4SA was secreted from *Enterococcus mundtii* ST4SA. These bacteriocins terminated the *Listeria monocytogenes* in the gut of mice. This study showed that the above described bacteriocins played an anti-infective role in in vivo experiments (van Zyl et al. 2019). As a colonizing peptide, bacteriocins can accelerate the entry and leverage of a probiotic strain into the niche present in the gastrointestinal tract (Gillor et al. 2008; Dobson et al. 2012).

10.5 Designer Probiotics as a Target Specific Tumor Knockout Therapy

Bacteria can be seen as an option for the treatment of the cancer (Hoffman and Zhao 2014). Scientists have reported diverse anaerobes specially in the solid tumor (Si et al. 2010) which inspires the application of microorganism as a vehicle for antineoplastic agents. Generally, strains from *Escherichia, Salmonella, Clostridium,* and *Bifidobacterium* may be used as designer probiotics for the treatment of cancer (Chua et al. 2017). *Bifidobacterium longum* exhibits magnificent potential to grow and reproduce in solid tumor (Kimura et al. 1980) and also has the potential to induce a powerful immunological response (Ashraf and Shah 2014). Proposed mechanism of action of designer probiotics as an anticancer therapy is shown in Fig. 10.3.

Bifidobacterium longum expresses tumstatin, which is angiostatin that hindered the proliferation and elicits programmed cell death in vascular endothelial cells inside tumors (Wei et al. 2016). Wei et al carried out studies, in which one group of mice treated with engineered *B. longum* and another is of control mice group (untreated). The results showed that relative decrease in the tumor metastasis and also higher viability was observed in the case of treated mice (Wei et al. 2016). Among the anaerobic probiotics, *Bifidobacterium* is versatile, because of its role in immune intervention, prophylaxis of cancer and infection (Karlsson et al. 2002; Ventura et al. 2014). Hence, *Bifidobacterium* has the most prominent use in probiotics. As *Bifidobacterium* is anaerobic, it is in favor of oxygen deprived region. Hypoxia occurs due to less availability of oxygen. Due to its anaerobic nature, it can be used as targeted cancer gene therapy for hypoxic environment of solid tumor

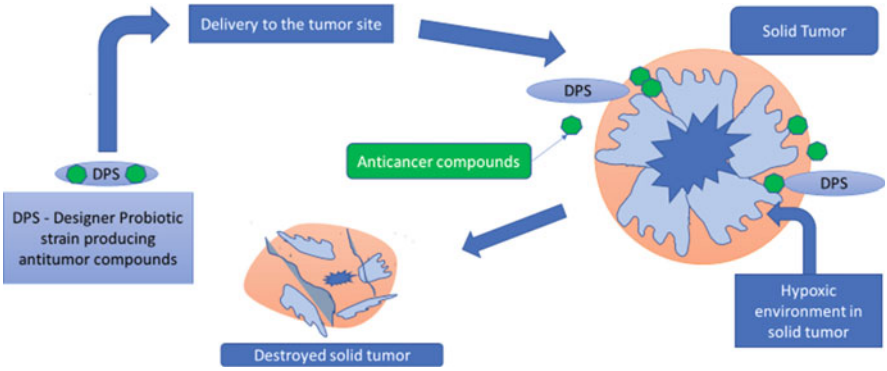

Fig. 10.3 Proposed mechanism of action of designer probiotic against cancer. (Designer probiotic which can grow into the hypoxic environment is constructed to secrete anticancer compounds at tumor surroundings)

(Cronin et al. 2012; Li et al. 2003; Tang et al. 2009; Yazawa et al. 2001; Yin et al. 2014; Zhou et al. 2016).

Oral route of administration is the most convenient route of administration. Hence, protein molecules and other pharmaceutically biological active molecules should be administered by this route. But the problem with the oral delivery is the proteolytic breakdown and denaturation in the gastrointestinal tract by various proteolytic enzymes. Lactic acid bacteria are the most favorable delivery carrier for proteins and biological drugs. The novel therapy introduced in the anticancer is the use of peptide called KiSS peptin, which plays a critical role to arrest the metastasis of cancer. Zhang et al carried out studies regarding expression in lactic acid bacteria and activity against human cancerous cells. KiSS1 gene was cloned into the vector (nisin inducible). These recombinant vectors were transformed into *Lactococcus lactis* NZ9000 cells. After induction with nisin the recombinant strain significantly secreted KiSS1 peptide. KiSS1 peptide induced inhibition of proliferation and migration of HT-29 cells. KiSS1 peptide is responsible for inhibition of proliferation and migration of human HT-29 cells by conciliating apoptosis and by decreasing expression of MMP-9 (Matrix Metalloproteinase- 9). It may be possibly a new strategy for cancer therapy (Zhang et al. 2016).

Lian et al reported the study of effect of recombinant *Escherichia coli* Nissle 1917 containing p53 and Tum-5 gene on the human hepatocellular carcinoma SMMC cell lines in BALB/c mice. p53 is responsible for tumor suppression, cell apoptosis, and cell cycle arrest (Hager and Gu 2013). Tum-5 is the anti-angiogenic protein responsible for the inhibition of formation of new blood vessel network called as neovascularization inside the area of tumor (He et al. 2017). Due to merging capability of p53 and Tum-5 to persuade apoptosis and anti-angiogenic activity, respectively, this can be the possible gene therapy for cancer. Tumor selective attack of *Escherichia coli* Nissle 1917 was studied by using luciferase L4XCDABE operon. Results showed that *Escherichia coli* Nissle 1917 could particularly target

the solid tumor region of SMMC-7721 cells tumor present in BALB/c nude mice. Fusion gene was constructed and delivered to solid tumor areas with the help of selective transporter *Escherichia coli* Nissle 1917 for cancer therapy. Three bacteria were bioengineered with Tum-5, p53, Tum5-p53 expressing gene, named as EcN (Tum5), EcN(p53), EcN (Tum5-p53) and used to investigate safety and efficacy. These studies also indicate that the combined protein EcN(Tum5-p53) has significantly more antitumor effect than single protein from EcN(Tum5) and EcN(p53) (He et al. 2019).

10.6 Designer Probiotics in HIV Infection

To obtain positive result of designer probiotics against microbes, they should withstand in the gut microbiota or reproductive tract to show the effect. They should also express and secrete antiviral compounds in sufficient portion and higher potency against viral/HIV infections. Pathogenicity is the main problem to use live microbes as therapeutics. Hence, microbes should be nonpathogenic. These are mandatory requirement for success of the live microbe use as a therapeutic (Rao et al. 2005).

Rao et al carried out studies regarding the use of peptides produced from bioengineered bacteria for the prevention against HIV infection. *Escherichia coli* Nissle 1917 used as a probiotic strain having ability to colonize in the intestine mucosa efficiently. HIV-gp41-hemolysin hybrid peptide responsible for the obstruction of HIV fusion and hindered access into the cell is cloned and expressed in *Escherichia coli* Nissle 1917. In vivo studies were carried out on mice. Recombinant *Escherichia coli* Nissle 1917 were efficiently replicating in the murine intestine for longer period up to certain months using antibiotic for selection. Colon microscopic studies prove the existence of abundant growth of bacteria and attachment to the mucus layer. Based on the results, they have concluded that there are certain advantages to use this technique for HIV treatment (Rao et al. 2005).

Petrova et al. carried out studies regarding the use of recombinant *Lactobacillus rhamnosus* GG and GR-1 probiotics against HIV. There are certain lectins such as actinohivin (AH) and griffithsin (GRFT), which can be used against HIV. For gastrointestinal tract route administration, recombinant *Lactobacillus rhamnosus*GG and for vaginal route of administration, recombinant *Lactobacillus rhamnosus*GR1 were used. For AH and GRAFT gene construct, Msp1 protein as a promoter was used. Genes responsible for AH and GRAFT expression extracellularly were attached to peptide of Msp1/p75 originated from *Lactobacillus rhamnosus*GG. There was failure of gene construct responsible for expression of AH monomers and dimers due to cellular toxicity. Recombinant strains constructed for the production of GRFT intra and extracellularly by *Lactobacillus rhamnosus*GR1 were successful. They had illustrated anti-HIV activity versus M tropic and T tropic HIV-1 strain. Anti-HIV lectins expressed by the engineered probiotic *Lactobacillus* can be used in future against HIV infections (Petrova et al. 2018). Various examples of clinical applications of designer probiotics are enlisted in Table 10.3.

10.7 Safety Concern of Designer Probiotics

The use of foreign bacteria in human body can give some unwanted effects that may or may not be predicted. Some of them are irreversible or incurable or may be lethal. Hence extensive study regarding safety is necessary. Before using genetically engineered probiotics the safety and biological containment concerns of probiotics should be addressed. In human gut, microbes are susceptible to gene transfer from one to another. Evaluation and extensive study regarding safety of microorganism should be done to prove its safe use in humans. Extensive phenotypic and genomic screening is necessary for potential candidate. These screening help to understand any existence of virulence factor or transferable genetic component (Sola-Oladokun et al. 2017).

Potential candidate for designer probiotic should be extensively screened for safety, pathogenicity, virulence activities before getting approval for human use. The potential candidate should not contain any antibiotic resistance gene which can be transferred (Amalaradjou and Bhunia 2013).

If any designer probiotic candidate contains antibiotic resistance gene, then it can be potentially transferred to natural microflora of human gut leading to antibiotic resistant microbes (Imperial and Ibana 2016).

In human gut, microbes are present in cell to cell contact and in close contact with each other, which may lead to the gene transfer (Jeong et al. 2019). Designer probiotic should not survive in the environment. The strain should not tolerate environmental stress. They should be incapable of dividing itself. Synthetic biology biocontainment method can be used as tool to develop bacterial kill switch which can be dependent on specific nutrient which is unavailable in environment. This can help to control the growth in the environment (Aggarwal et al. 2020). Engineered probiotics need to produce their effect on target site. Biomarkers play important role in gut inflammation diagnosis as well as in achieving precise treatment (Barra et al. 2020).

For evidence of therapeutic efficacy in vitro models should be developed. These in vitro models can mimic the environment present in the body. In vitro and also in vivo models' study can give relevant data regarding efficacy of probiotics, which can be used for desire therapeutic effect of probiotic candidate. Animal study is also important to check maximum dose and other toxic effects. Selection of animal is also critical that animal should have some similarity like humans (Charbonneau et al. 2020). Extensive clinical trials are necessary to use as therapeutic in human.

10.8 Future Perspective

Probiotics are microorganisms that remain in healthy association with human gut. Their use as a rejuvenation therapy to replenish the gut micro flora after extensive antibiotic treatment makes them suitable targets for further clinical applications. Designing of probiotics for effective GI tract targeting with absence of side effect is a challenging task. Various molecular biology techniques such as cloning, expression

Table 10.3 Applications of designer probiotics

Engineered probiotic	Strategies applied	Therapeutic Applications	Reference
Escherichia coli Nissle 1917	Enterocin A, Enterocin B and Hiracin JM79 production as AMPs, which particularly aimed and terminate *Enterococcus*	Decrease in the *Enterococcus* population resistant to vancomycin present in intestine	Geldart et al. (2018)
Lactobacillus reuteri 100-23C (pHENOMMenal)	Cloning and expression of phenylalanine lyase gene in *Lactobacillus reuteri*	Reduction in the quantity of phenylalanine in the blood	Durrer et al. (2017)
Escherichia coli Nissle 1917	As a vehicle to deliver p53 and Tum5 proteins specifically at oxygen deprived site of tumor	Hindered the proliferation and growth of human hepatocellular carcinoma SMMC-7721 cells	He et al. (2019)
Bifidobacterium recombinant thymidine kinase with ganciclovir (BF-rTK/GCV)	Inhibition of expression of VEGF (vascular endothelial growth factor)	Stopped tumor angiogenesis	Zhou et al. (2016)
Lactobacillus rhamnosus GG and *L. rhamnosus* GR-1	Anti-HIV lectins, actinohivin, and griffithsin have activity against an M-tropic and a T-tropic HIV-1 strain	Inhibition of HIV transmission and replication	Petrova et al. (2018)
Lactococcus lactis NZ9000 strain	Expression of human PAP in *Lactococcus lactis* has therapeutic effect in murine DNBS (dinitrobenzene sulfonic acid) induced colitis	Prevention of intestinal mucositis caused due to chemotherapy medications	Carvalho et al. (2017)
Lactobacillus sakei	The recombinant microbe can be used as protective therapy due to which increased dose of radiation can be possible	Therapy for enteritis caused due to radiation	He et al. (2018)
Lactococcus lactis	Cloning and expression of TRAIL (tumor necrosis factor related apoptosis inducing ligand) protein in *Lactococcus lactis*	TRAIL protein promotes programmed cell death in human adenocarcinoma and colon carcinoma	Bohlul et al. (2019)

are discussed in detail in this review used for designing probiotics with an extensive selection of bacteria as a pre-requirement. Probiotics are becoming popular day by day due to their health effects on human body. Due to the modern recombinant DNA technology, designer probiotics can be used in the prevention and treatment of various diseases. There are many microbes discovered as probiotics and can be used to design their DNA by incorporating desired gene which can secrete antimicrobial substance or peptide or anticancer substances. Designer probiotics are the

modern tool to counteract various diseases such as diarrhea, dysentery, Crohn's disease, cancer, diabetes. Due to the modern genetic engineering tools probiotics can be transformed as designer probiotics which can become survive at extreme conditions. From the many studies, it may be said that designer probiotics can be used as therapeutics. The safety of the designer probiotics is the problem. Mutation can occur in a microbial cell which may regain its virulent activity. It is challenging to produce robust probiotics. There is tremendous growth in the recombinant DNA techniques, which can help to build robust probiotics.

Designer probiotics can be used as targeted therapy for certain diseases. They can be used as a vehicle to deliver cytotoxic compounds at the site of tumor, which decreases the side effect. They have versatile uses in the treatment of enteric diseases when used as receptor mimic strategy. Designer probiotics can overcome the disabilities of probiotics in terms of therapeutic effect. As the treatment of cancer needed specificity to avoid side effects, many researchers are studying on the microbes which are anaerobes and particularly grow in hypoxic region of solid tumor. By incorporating anticancer compounds or biochemicals we can target solid tumor with the help of designer probiotics. These type of treatments need excessive study from the point of safety and other parameters. As we can see already probiotics can secrete antimicrobial compounds which help to survive them. We can also incorporate different types of antimicrobial peptides expressing genes to increase the potency against pathogens. Techniques like synthetic biology, genetic engineering techniques, and metabolic engineering can improve the robustness and effectiveness of probiotics. The modern era is now studying genetics extensively, which can help to solve many problems. Extensive research is needed to do in future to use designer probiotics as a therapeutic. Clinical trials should be carried out to study, monitor, and report effects of designer probiotics. Clinical trials are necessary to use designer probiotics in humans.

10.9 Conclusion

The risk of pathogens causing enteric infections is increasing day by day. Deaths due to enteric infections are also elevated due to lack of certain treatment. Probiotics can improve this scenario. Their use has been implicated to improve digestion and other stomach related problems. But conventional probiotics have some disadvantages like lack of specificity, stress tolerance, and viability conditions. Designer probiotics can overcome these problems. They are genetically modified probiotic microorganism intended to therapeutic use. Many scientists and research groups are working on designer probiotics. Regulatory requirements are also important to use designer probiotics as a therapeutic. We need to develop certain criteria to fulfill regulatory requirements. Safety is also an important concern in case of use of designer probiotics. Due to the use of genetically modified organism safety and regulation of them are important. Modern molecular biology techniques and genetic engineering, synthetic biology techniques have more application in development of designer probiotics. There are tremendous applications of designer probiotics in enteric

infections, cancer, HIV, food borne infection, immunity related disorders, etc. They can be the option for targeted therapy in many disease conditions. These types of probiotics should be surviving in effective amount to give their beneficial effects in host. So, robust model of designer probiotics need to be developed for survival. The effect of designer probiotics on the gut microflora needs to be studied extensively. Critical and stringent assessment is necessary to use designer probiotics therapy in humans.

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Probiotic Interventions for Oral Health

11

Svante Twetman and Mette Rose Jørgensen

Abstract

The evolving understanding that the oral diseases are preventable by modulation of the oral biofilm has paved the way for the use of beneficial bacteria in dentistry. The main oral diseases, dental caries and periodontitis, result from an ecological shift of the health-associated commensal microbiota to a dysbiotic oral biofilm with abundance of acid-tolerating or proteolytic bacteria. In this chapter, we review the current evidence for the role of probiotic bacteria in the prevention and management of oral diseases. There is evidence that probiotic bacteria can prevent early childhood caries when administrated with milk or tablets in daycare settings. For school children and adults, the certainty of evidence is low due to few and small studies with risk of bias. For periodontal diseases, lactobacilli-derived supplements clearly display significant clinical improvements on gingival bleeding and pocket depth when combined with the conventional treatment modalities. Probiotic supplements may also reduce the need of antiseptics and antibiotics in periodontal care. Likewise, probiotics can significantly lower oral *Candida* counts, ameliorate bad breath and improve oral wound healing. In summary, probiotics play an increasing role in the prevention and comprehensive management of oral diseases, but we need more long-term studies, especially

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with candidate strains isolated from the oral cavity. In addition to the clinical efficacy, health-economic aspects should be addressed. The development of oral synbiotic products, containing both pre-and probiotics, is of interest for future oral health care.

Keywords

Probiotics · Oral health · Periodontal disease · Oral medicine · Peri-implant disease

11.1 Introduction

In the early 1990s, Russian scientists first described the adoption of probiotics for oral health but, at that time, the general interest was low. With the evolving understanding of the oral microbiome and its critical role in maintaining oral health, the concept has currently gained attention and momentum. The composition of the oral biofilm is unique for each individual and modified by genetics, order, and timing of exposure to microbes, diet, and location within the oral cavity. Most bacteria are located on the tongue and the oral mucosa while approximately 20% constitute the dental plaque (Kilian et al. 2016). The dynamic biofilm contains many different types of bacteria with various properties and functions (Marsh 2018). A rich, diverse, and balanced biofilm in symbiosis with the host is associated with health and acts as a fluoride reservoir and a protective barrier to erosion. The major oral diseases, caries and periodontitis, are classified as non-communicable diseases rather than classical infections (Twetman 2018). An ecologically driven shift of the commensal microbiota from symbiosis to dysbiosis (Fig. 11.1) will increase the risk of caries, periodontal diseases, or oral candidiasis (Kilian et al. 2016). The individual mix of functional microbial clusters in the oral biofilm is, however, important to keep in mind as people differ in stress tolerance and biofilm resilience. Recent research suggests that individuals with a "saccharolytic" ecotype of their salivary microbiome seem more vulnerable for sugar exposure and subsequent caries development while others have a "proteolytic" ecotype and, thereby, are more prone to developing inflammatory-induced periodontal diseases (Zaura et al. 2017). Consequently, regular ingestion of pre- and probiotics to support the maintenance of a healthy oral biofilm (primary prevention) or to restore a dysbiotic biofilm (secondary prevention) is an emerging treatment option within dentistry (Cummins and Marsh 2018). The dominant vehicles of administration are dairy products and tablets/lozenges, but specific oral care products such as probiotic rinses and dentifrices are available in some countries. This chapter will briefly summarize the role and evidence of probiotic bacteria in preventive dentistry from a clinical perspective. A general remark is that the probiotic strategy is an adjunct rather than an alternative to the established evidence-based technologies and/or good clinical practice.

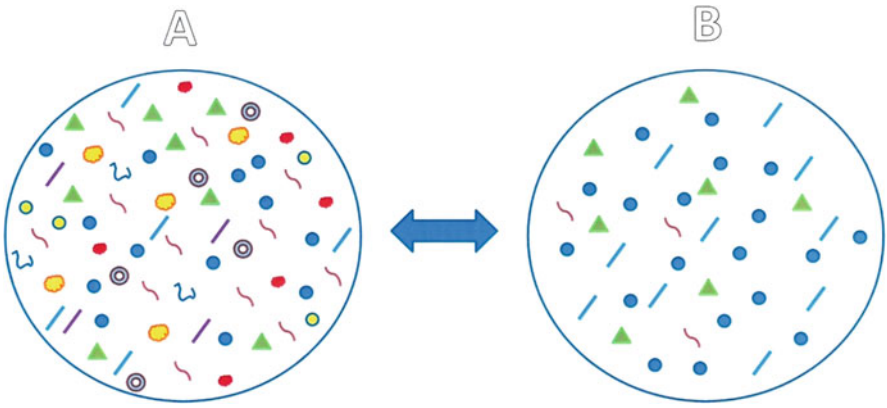

Fig. 11.1 Illustration of the ecological shift from a rich and diverse symbiotic oral biofilm (**a**) to a dysbiotic (**b**) state with reduced diversity and abundance of acidogenic acid-tolerant (caries-associated) or proteolytic (associated with periodontal disease) species. The process can go both ways: by dealing with the drivers of dysbiosis and exposure to beneficial bacteria, a stable health-associated biofilm may be re-established. Drawing by Eva Marie Reinwald

11.2 Mechanisms Involved

It is generally thought that the effect of probiotic bacteria is genera and strain specific (de Vrese and Schrezenmeier 2008). The most common strains applied for oral health are presented in Table 11.1. The exact mechanisms of probiotic action need to be further unveiled but there is evidence for a series of combined local and systemic events illustrated in Figs. 11.2 and 11.3. The local biofilm effects rely on (1) bacterial co-aggregation, (2) bacteriocin and hydrogen peroxide production, (3) competition for adhesion sites, and (4) competition for nutrients (Reid et al. 2011). The systemic gut-mediated effects are well known, and the immunomodulation is expressed along the gut–oral cavity axis via saliva and gingival crevicular fluid (GCF). For example, we and other research groups have demonstrated elevated concentrations of salivary IgA in connection with intake of probiotic lactobacilli (Braathen et al. 2017; Pahumunto et al. 2019) and a significant downregulation of pro-inflammatory cytokines (TNF-alpha and IL-8) in GCF (Twetman et al. 2009). Thus, there are proofs of principle that the probiotic approach can modulate inflammation in the oral cavity. It is also clear that the consumption of beneficial bacteria in dairy products, in particular milk and yogurt, is helpful against acid biofilm stress since such intake is associated with increased pH levels in the oral environment (Nadelman et al. 2018; Villavicencio et al. 2018).

The pertinent question is whether a regular intake of probiotic bacteria can influence the amount and composition of the oral microbiota. Firstly, mouth rinses containing probiotics seem to be equally effective as oral chlorhexidine rinses to control the amount of dental plaque when used by schoolchildren after tooth brushing (Kandaswamy et al. 2018; Shah et al. 2019). Secondly, there is strong

Table 11.1 Example of probiotic bacteria used in clinical studies for oral health

Probiotics	Caries	Gingivitis	Periodontitis	Peri-implantitis	Candidiasis	Halitosis
L. salivarius	X		X			X
L. rhamnosus	X		X		X	X
L. acidophilus					X	
L. casei		X				
L. paracasei	X					
L. reuteri	X	X	X	X	X	X
L. brevis		X	X			X
L. bulgaricus					X	
L. plantarum			X			
L. paracasei	X					
B. lactis	X	X				
B. longum					X	
B. brevis	X					
S. thermophilus					X	
S. oralis	X	X	X			
S. uberis	X	X	X			
S. rattus	X	X	X			
S. salivarius	X					

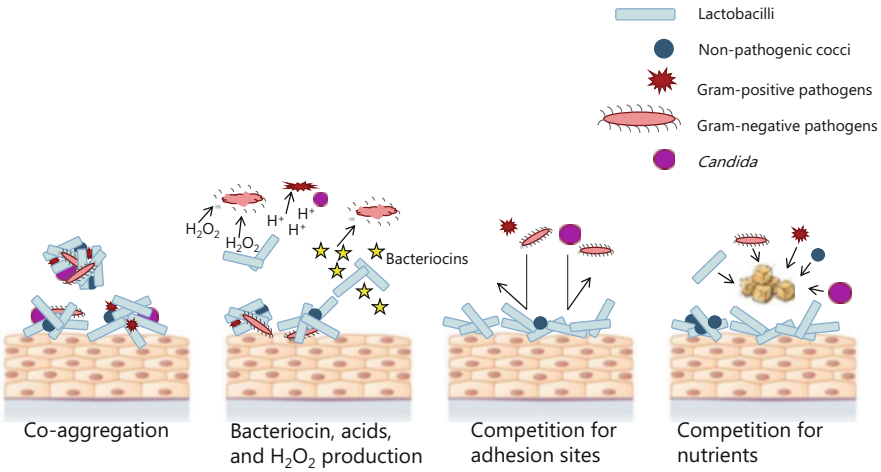

Fig. 11.2 Local/direct mechanisms in the oral biofilm induced by probiotic bacteria. Drawing by Mette Rose Jørgensen, based on Reid et al. (2011)

evidence from systematic reviews that interventions with probiotic bacteria result in significant reductions of caries-associated mutans streptococci and major periodontal pathogens such as *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum,* and *Porphyromonas gingivalis* in saliva and dental plaque (Cagetti et al. 2013; Laleman et al. 2014; Gruner et al. 2016; Matsubara et al. 2016). However, a common observation is that the utilized probiotics are only transient colonizers (1–2 weeks) of the oral cavity and that the long-term impact of probiotics on the composition of the oral microbiome is a knowledge gap. Two studies have shown an increased diversity and a transient shift to common commensals in saliva on the expense of reduced levels of pathogens (Dassi et al. 2014; Romani Vestman et al. 2015). Other researchers have failed to show any major impact on the oral microbiome after short-term interventions with probiotic strains from the *Bifidobacterium* and *Lactobacillus* family (Toiviainen et al. 2015; Keller et al. 2018). Obviously, we need more data to elucidate the influence of beneficial bacteria on the composition and function of the oral microbiome. It is, however, important to emphasize that no severe adverse events or side effect has hitherto been reported in connection with any clinical trial performed in the context of oral health.

11.3 Clinical Effects

11.3.1 Probiotics and Dental Caries

Dental caries is a biofilm-mediated, sugar-driven, multifactorial, dynamic disease that results in the phasic demineralization and remineralization of dental hard tissues (Pitts et al. 2017). In fact, it is the world's most common disease affecting over three

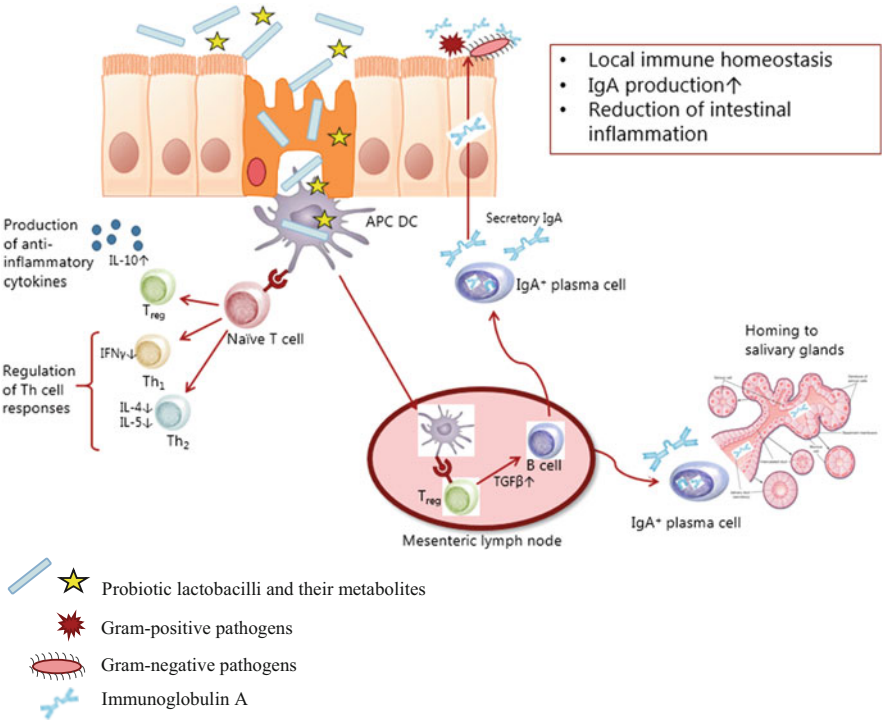

Fig. 11.3 Systemic immunomodulation in the gut induced by probiotic supplements. Drawing by Mette Rose Jørgensen. The local immune system in the gut reacts less viciously towards commensal/probiotic bacteria compared with pathogenic bacteria. In specialized immunological compartments, called Peyer's Patches, antigen presenting Dendritic Cells (APC DC) are only slightly stimulated through specialized M cells. DCs present antigens to Naïve T cells which in turn differentiate to regulatory T cells (Treg) that produce anti-inflammatory cytokines, such as interleukin-10 (IL-10), and to T helper (Th1) and (Th2) cells, that secrete lesser amounts of pro-inflammatory and pro-proliferatory cytokines resulting in a weaker immune response. In the mesenteric lymph nodes, the activated DCs present antigens to naïve T cells that differentiate to Treg cells that produce Transforming Growth Factor beta (TGF-beta). This leads to the differentiation of B-cells to immunoglobulin A (IgA) producing plasma cells, that migrate back to the lamina propria in the gut and secrete dimeric IgA or migrate to distant sites such as the salivary glands where they secrete IgA via receptor mediated endocytosis

billion people worldwide (Marcenes et al. 2013). In the recent decades, a shift has occurred in the management of caries, from the traditional restorative approach with fillings to non-operative or minimally invasive strategies (Pitts et al. 2017). In this context, probiotic supplements have emerged in parallel to the existing strategies for primary and secondary prevention; daily fluoride exposure, sugar reduction, and regular mechanical disruption of the oral biofilm (Twetman 2018).

Several clinical trials have employed probiotics to prevent early childhood caries in preschool children, crown caries in adolescents, and root caries in older adults. In a systematic review, Zaura and Twetman (2019) compiled the results from 12 studies

Table 11.2 Caries preventive effect of probiotic therapy obtained in clinical trials. Data from Zaura and Twetman (2019)

Age group	Number of studies	Caries preventive effect[a]	P^b
Preschool children	5 studies; 1273 children	RR 0.65 (95% CI 0.47–0.90)	<0.05
Early-in-life probiotics[c]	3 studies; 474 children	RR 0.68 (95% CI 0.42–1.09)	NS
Schoolchildren	3 studies; 182 children	RR 0.70 (95% CI 0.45–1.09)	NS
Elderly	1 study; 80 older adults	RR 0.61 (95% CI 0.46–0.81)[d]	<0.05

[a]Caries prevalence (dichotomized as yes vs.no) and expressed as risk ratio (RR) with 95% confidence interval
[b]*NS* not statistically significant
[c]Intervention was directed to toddlers, caries was scored in the primary and permanent dentition
[d]Root caries reversal (dichotomized as yes vs. no) and expressed as risk ratio (RR) with 95% confidence interval

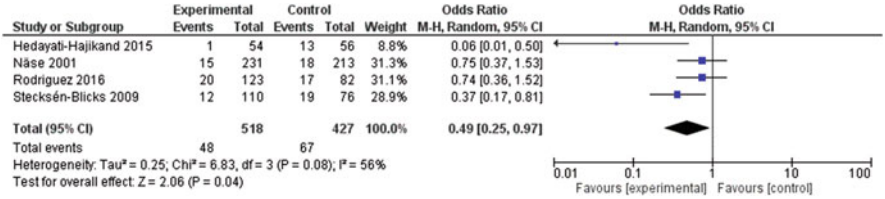

Fig. 11.4 Pooled Odds Ratio for the incidence of early childhood caries in randomized controlled trials with probiotic bacteria in milk or lozenges (experimental) vs. placebo (control) in daycare settings for 12–21 months. The caries preventive effect was statistically significant (OR 0.49; 95% CI 0.25, 0.97). Hedayati-Hajikand et al. (2015), Näse et al. (2001), Rodríguez et al. (2016)

and a summary of the main results is presented in Table 11.2. The most outstanding finding was that probiotic interventions conducted in daycare or nursery school settings resulted in significantly reduced caries increment in the primary dentition. The pooled odds ratio calculated from four randomized controlled trials with a duration of 12–21 months was 0.49 (95% CI 0.25–0.97; $p < 0.05$) as illustrated in Fig. 11.4. The predominant vehicle for the probiotics given to the preschool children was milk (fresh or powder) supplemented with probiotic *Lactobacillus* strains. Daycare personnel supervised the daily administration, which secured a good compliance. Notably, most of the trials were conducted in low socioeconomic and/or rural areas with elevated caries risk and the probiotic approach contributed to reduce inequalities in dental health. The participants in both the test and control groups were supplied with and strongly encouraged to use fluoride toothpaste so the reduced incidence of caries was additive to the anti-caries effect of fluoride. Another interesting parallel finding was that significant improvements in general health were reported from three of the caries-trials in terms of reduced respiratory tract infections, reduced prescription of antibiotics, reduced days with sick leave, and reduced incidence of IgE-associated eczema (Hatakka et al. 2001; Stecksén-Blicks et al. 2009; Taipale et al. 2016; Twetman et al. 2017). Such health benefits are not only important for the individual child but also for their parents and the society.

Health-economic analyses should therefore be included in future trials with probiotic interventions in dentistry.

Three studies were post-trial evaluations; the probiotic intervention took place early in life with medical endpoints, but the effect on caries prevalence was scored in primary and permanent teeth several years thereafter (Hasslöf et al. 2013; Taipale et al. 2013; Stensson et al. 2014). The results were mixed due to different kinds of study bias as discussed by Zaura and Twetman (2019). The caries preventive effect in schoolchildren was unreliable but statistically significant when only those with high caries risk were considered (Teanpaisan et al. 2015). One single study addressed the reversal of primary root caries lesions in older adults after a 14-month daily intake of milk supplemented with probiotic lactobacilli (Petersson et al. 2011). The test group showed significantly more reversals, verified by visual scoring and electric resistance measurements, when compared with the control group consuming milk without probiotic bacteria. This may be significant in the future, since root caries is a growing problem globally and demanding to treat. To summarize, with the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) system of rating evidence (Guyatt et al. 2008), the certainty of the effect estimate is low to moderate for early childhood caries prevention but very low for school children and adults due to the limited numbers of trials, risk of bias, and partly inconsistent results.

11.3.2 Probiotics and Periodontal Disease

The most investigated "probiotic domain" in dentistry is the effect of *Lactobacillus*-derived supplements on inflammatory conditions in the periodontal tissues. Periodontal diseases (gingivitis and periodontitis) are prevalent in both developed and developing countries, affecting around 20–50% of the global population (Marcenes et al. 2013). The diseases occur when a susceptible host is challenged by an increasing burden of proteolytic bacteria in the biofilm. Gingivitis is a biofilm-related swelling, localized to the gingiva while periodontitis is characterized by attachment loss, alveolar bone destruction, and bone loss. Scaling, root planing, and debridement of deep pockets are the gold standard for patients with chronic periodontitis but adjunct therapies, such as local antiseptics and antibiotics, are advocated when the progression is aggressive. To study the effectiveness of clinical interventions, plaque index, gingival bleeding index, bleeding on probing (BOP), pocket probing depth (PPD), and clinical attachment level (CAL) are typical endpoints. The recent systematic reviews and meta-analyses on probiotic supplements and periodontal disease are listed in Table 11.3 together with the main conclusions. A common observation was that the beneficial effects of the probiotic therapy were greater than expected considering the minimal impact on the oral microbiota; the treatment improved the clinical signs of chronic and aggressive periodontitis in a significant way. For example, a meta-analysis of three studies showed that the probiotic intervention reduced the periodontal pockets with an average of 0.5 mm and the BOP by 15% (Martin-Cabezas et al. 2016). Two recent

Table 11.3 Recent systematic reviews (SR) of randomized controlled trials (RCT) on the use of probiotics for the prevention and management of periodontal disease

First author, year (type of review)	No. of RCTs	Authors' conclusions
Gruner, 2016 (SR and meta-analysis)	10 studies	Evidence is supportive towards managing gingivitis and periodontitis with probiotics
Matsubara, 2016 (SR)	12 studies	Oral administration of probiotics is a safe and effective adjunct to conventional mechanical treatment in the management of periodontitis, especially the chronic disease entity
Martin-Cabezas, 2016 (SR and meta-analysis)	4 studies	Findings support the adjunctive use of *L. reuteri* to scaling and root planing in chronic periodontitis treatment at short-term, especially in deep pockets
Jayaram, 2016 (SR)	13 studies	Probiotics in the treatment of periodontal disease produce short-term clinical and microbiologic benefits
Ikram, 2018 (SR and meta-analysis)	7 studies	Adjunctive probiotics (*L. reuteri*) could result in additional benefits in clinical attachment level gain in chronic periodontitis
Vives-Soler, 2020	9 studies	Probiotics may provide an additional benefit to manual debridement in chronic periodontitis.

studies have reinforced these findings by employing *Lactobacillus reuteri*; Grusovin et al. (2019) showed a clear CAL improvement in patients with severe/advanced periodontitis and Laleman et al. (2020a) demonstrated a significant beneficial impact on residual pockets remaining after conventional treatment. A common observation from the periodontal trials is that the positive effects of probiotics are due to the modulation of the host response, not the anti-plaque effect. Additional claims from several studies were that the probiotic intervention could reduce the need for periodontal surgery and replace antibiotics in the management of periodontal infections (Martin-Cabezas et al. 2016; Matsubara et al. 2016; Gruner et al. 2016; Ikram et al. 2018; Laleman et al. 2020a). The systematic reviews highlighted that a continuous probiotic administration was necessary to maintain these benefits although long-term studies were lacking. In summary, the evidence is supportive for the use of probiotic *Lactobacillus* strains in addition to manual debridement in the management of gingivitis and chronic periodontitis. However, more studies are required on dose, route of administration, and type of probiotics used.

11.3.3 Probiotics and Peri-Implant Diseases

The most common reasons for implant failure are inflammation and occlusal overload. Inflammation in the soft tissues around the implant without bone loss is termed peri-implant mucositis while the presence of inflammation with marginal peri-implant bone loss is described as peri-implantitis. The average prevalence of mucositis and peri-implantitis is 43% and 22%, respectively (Derks and Tomasi

2015). The treatment with non-surgical debridement is similar to that of periodontitis but the outcome is less favorable, and the clinical situation is often not completely resolved. The adjunctive use of probiotics has provided inconsistent results. In three studies, the administration of a daily lozenge of *Lactobacillus reuteri* containing at least 200 million live bacteria for 30 days, together with mechanical debridement, improved the clinical parameters around implants with mucositis and/or peri-implantitis over a 90-day period (Flichy-Fernández et al. 2015; Tada et al. 2018; Galofré et al. 2018). Other researchers found clear probiotic-induced clinical enhancements although not significantly better than placebo (Hallström et al. 2016; Mongardini et al. 2017). An interesting observation was that the clinical improvements took place despite a very limited effect on the peri-implant microbiota (Laleman et al. 2020b). Thus, it seems reasonable to assume that probiotics prevent inflammation mainly by affecting the host response rather than by affecting selected pathogens in patients affected by peri-implantitis (Tada et al. 2018). The certainty of evidence is very low and further studies addressing the probiotic effects on the *microbiome adjacent to titanium implants* are required to expand the knowledge of this specific ecosystem.

11.3.4 Probiotics and Oral Medicine

11.3.4.1 Oral Candidiasis

Candida albicans is the most prevalent *Candida* spp. in humans and around 50% of the healthy population harbor its neutral blastospore form in the oral cavity (Sardi et al. 2013). *Candida* spp. interact physically with bacteria by co-aggregation and can be seen in the "corncob" structures of the dental biofilm. Under certain circumstances, the homeostatic state of the bacteriome-mycobiome biofilm is disrupted, and the *Candida* spp. become pathogenic and cause infection in the oral mucosa, termed oral candidiasis (Oever and Netea 2014). Predisposing factors are immune-compromised individuals, broad-spectrum antibiotic treatment, hyposalivation due to disease or polypharmacy, neglected oral hygiene, extensive use of corticosteroids, wearing dentures, and smoking (Pankhurst 2012).

Based on pre-clinical findings, probiotic therapy seems to prevent and combat oral candidiasis; in vitro studies have shown inhibition of *C. albicans* growth by lowering pH and by production of bacteriocins and hydrogen peroxide (Hasslöf et al. 2010; Ujaoney et al. 2014). Furthermore, Ujaoney et al. (2014) proved that the supernatants and bacterial suspensions of commercially available probiotics containing *Lactobacillus* spp. reduce the in vitro ability of *C. albicans* to form biofilms on dentures. Jørgensen et al. (2017) showed that probiotic lactobacilli co-aggregate with various *Candida* species, and inhibit growth via production of H_2O_2 and organic acids. In parallel, clinical trials have evaluated the antifungal effects of probiotic supplements in the oral cavity. A summary of published randomized controlled trials is presented in Table 11.4. A pioneering study published by Hatakka et al. (2007) showed a significant reduction of high yeast counts in the saliva of elderly individuals after daily consumption of probiotic cheese

Table 11.4 Summary of randomized controlled clinical trials examining the antifungal effects of probiotics in the oral cavity

First author, year	Probiotic strains	N/population/vehicle/time	Results
Lee, 2019	*L. rhamnosus* SP1	36/denture wearers/milk/12 mo	Significant reduction in severity of denture stomatitis and significantly reduced *Candida* counts in probiotic group
Hu, 2019b	*S. salivarius* K12	56/oral candidiasis/lozenges as adjuvant to nystatin/4 wk	Significantly improved cure rate in probiotic group
Miyazima, 2017	*L. acidophilus* NCFM *L. rhamnosus* Lr-32	60/denture wearers/cheese/8wk	Significant reduction of *Candida* levels in probiotic groups
Mishra, 2016	*S. oralis* KJ3 *S. uberis* KJ2 *S. rattus* J	60/children, 6–14 years/rinse/1wk	Probiotics equally effective as chlorhexidine 0.2% rinse in reducing *Candida* counts
Kraft-Bodi, 2015	*L. reuteri* ATCC PTA 5289 *L. reuteri* DSM 17938	215/frail elderly/lozenges/12 wk	Significant reduction in high yeast counts (CFU/ml) in saliva and plaque in probiotic group
Ishikawa, 2015	*L. rhamnosus* HS111 *L. acidophilus* HS101 *B. bifidum*	59/denture wearers/capsules/5wk	Significantly reduced detection rate of *Candida* spp. in probiotic group
Li, 2014	*L. bulgaricus* *B. longum* *S. thermophilus*	65/patients with *Candida*-associated stomatitis/lozenges/4wk	Significantly reduced detection rate of *Candida* spp. and symptom relief in probiotic group
Hatakka, 2007	*L. rhamnosus* LC705 *L. rhamnosus* GG *P. freudenreichii* ssp. *shermanii* JS	276/elderly/cheese/16wk	Significant reduction in high yeast counts (CFU/ml) in saliva in probiotic group
Ahola, 2002	*L. rhamnosus* GG *L. rhamnosus* LC705	74/young adults/cheese/3wk	Significant reduction of yeast counts in probiotic group

S. Streptococcus, *L. Lactobacillus*, *B. Bifidobacterium*, *P. Propionibacterium*, *wk* intervention time in weeks, *mo* intervention time in months, *CFU* colony forming units

containing four different probiotic spp. and similar findings were later confirmed by other researchers (Li et al. 2014; Ishikawa et al. 2015). Thus, the probiotic anti-*Candida* properties seem particularly suitable for older adults as demonstrated through reduced salivary and plaque counts of *C. albicans* in frail elderly living in nursery homes and among patients with dentures (Kraft-Bodi et al. 2015; Miyazima et al. 2017). The published research is compiled in two recent systematic reviews

(Mundula et al. 2019; Hu et al. 2019a) and a meta-analysis based on four trials in elderly has concluded that probiotic products have a preventive and suppressive effect on oral candidiasis (Ai et al. 2017). This is of importance in the light of an increasing occurrence of drug-resistance in *Candida* spp. due to overuse of antifungal medications, systemic toxicity, and cross-reactivity with other drugs.

11.3.4.2 Recurrent Aphthous Ulcers (RAU)

Aphthous ulcers are round or ovoid painful ulcers that commonly (5–25%) recur in the oral mucosa among children and young adults (Cui et al. 2016). The etiology is unclear, but the ulcers normally heal within 2 weeks. The treatment is symptomatic, mainly with aid of topically applied antiseptic agents, local anesthetics, and topical corticosteroids. A novel approach is to employ probiotic bacteria in order to alleviate the symptoms. A first placebo-controlled pilot trial has indicated that daily use of *Lactobacillus reuteri* lozenges could reduce the ulcer severity score (USS) in patients with RAU over a period of 3 months (Pedersen et al. 2019). The USS score considers six important lesion characteristics: number, size, duration, ulcer-free period, site, and pain. The concept merits further attention as a possible adjunct to the management of pain-affected patients with chronic and recurrent ulcers.

11.3.4.3 Halitosis (Bad Breath)

Oral malodor (halitosis) is a common condition affecting the psychological and social life of individuals. The main reason is the production of volatile sulfur compounds (VSC) such as hydrogen sulfide and methyl mercaptan from gram-negative bacteria located on the dorsum of the tongue. The basis of oral malodor control is regular mechanical removal and disruption of the oral biofilm combined with dentifrices and mouthwashes. The effect of probiotic supplements on oral malodor has been investigated in several placebo-controlled clinical trials with an organoleptic endpoint or by measuring the concentration of VSCs (Georgiou et al. 2018). A meta-analysis has shown that the organoleptic scores were significantly lower in subjects given probiotics (mainly *Lactobacillus* strains) for at least 2 weeks compared with placebo, but no significant differences were observed concerning the VSC concentrations (Yoo et al. 2019). It should, however, be underlined that the probiotic effects on oral malodor seem to be modest rather than dramatic. Although current evidence is supportive of recommending probiotics for the management of halitosis, the studies were of short duration and heterogeneous with respect to the intervention. Halitosis may also occur in association with severe periodontitis, and according to Soares et al. (2019) probiotics can be a valuable adjunct to the comprehensive management of this disease with a subsequent reduction in oral malodor. The probiotic mechanisms of action concerning halitosis must be further evaluated, as well as the question whether or not the organoleptic improvements are stable over time.

11.3.5 Probiotics and Maxillofacial Surgery

Recent studies have shown that strains of *Lactobacillus reuteri* can promote and enhance wound healing and may thereby be of potential interest for the surgical sciences (Twetman et al. 2018; Han et al. 2019). To our knowledge, only one clinical trial has so far brought the probiotic philosophy into oral and maxillofacial surgery. In a clinical trial, patients referred for surgical removal of impacted third molars (wisdom teeth) were randomly assigned to use lozenges containing two strains of *Lactobacillus reuteri* (DSM 17938 and ATCC PTA 5289) or placebo, three times daily during two post-operative weeks (Wälivaara et al. 2019). The results showed no significant effect on the clinical wound healing index scored by the surgeons, but the patients reported subjectively perceived differences with aid of an analogue VAS-scale. The self-reported data unveiled a significantly reduced sense of swelling, particularly during the second week after surgery in the probiotic group. Likewise, patients in the probiotic group had significantly fewer nights with disturbed sleep and fewer days with sick leave from work (Wälivaara et al. 2019). Since pain, swelling, and discomfort are almost mandatory complications after third molar removal, such patient-perceived post-operative ameliorations can be important drivers for the adoption of bacteriotherapy in dentistry.

11.4 Future Trends

For the future, we need to close the knowledge gap on the dose-response and optimal probiotic interference with oral biofilms. Additionally, the duration of the intervention and variations in the mode of delivery (dietary, self-, or professionally applied) needs to be mapped with a core outcome set of validated oral endpoints. The search for, and validation of, new probiotics strains belongs to the future (Keller et al. 2018). Most probiotic strains used for oral health are of gastro-intestinal origin, which may not be optimal for the unique and complex environment in the oral cavity. Recently, a commensal oral *Streptococcus* strain, *Streptococcus dentisani,* was isolated from supra-gingival plaque of healthy individuals (López-López et al. 2017). Due to its inhibitory action against *Streptococcus mutans,* arginolytic activity, and pH buffering capacity, *Streptococcus dentisani* holds the potential of a coming anti-caries probiotics (Ferrer et al. 2020). Another future challenge is to combine prebiotics and probiotic strains into one synbiotic consumer product. This seems of interest in dentistry since both prebiotic arginine and xylitol have caries-inhibiting properties (Nascimento 2018; Janakiram et al. 2017). Pre- and probiotics are natural and affordable food additives with small or even negligible risk of side effects. Thus, there is a reasonable chance that this prophylactic concept can be accepted by the profession, as well as by the patients/consumers, especially if it is health-economically favorable. The potential "spill-over" to other non-communicable conditions such as overweight, diabetes, and cardiovascular diseases may further enhance the awareness of probiotics among dental health professionals.

11.5 Conclusions

The uses of probiotic supplements to support health-associated oral biofilms and interfere with the drivers of dysbiosis have shown consistent improvements in oral health when applied as adjunct to the good clinical practice of oral diseases. An international consensus group has declared that "*probiotics could be helpful in caries prevention and periodontal disease management, although the biological mechanisms are not fully elucidated*" (Chapple et al. 2017). The best evidence is available for early childhood caries prevention while the evidence is low but suggestive for the periodontal diseases, oral candidiasis, and oral malodor. Further research on the mechanisms of action and clinical impact will provide better insight and help to strengthen the probiotic concept in dentistry.

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Probiotics Targeting Enteric Infections

12

Kavita Pandey and Jyoti S. Gokhale

Abstract

Globally, enteric infections represent one of the biggest challenges faced by humanity. According to the global burden of diseases study 2016, it has been estimated that diarrhoeal illness causes approximately 1.7 million deaths annually and results in 74 million disability-adjusted life years lost. Though antibiotic therapy is the most effective against enteric infections, antibiotic resistance represents the primary concern. The role of gut microbiota in retaining healthy life is well recognized as the concept of replenishing good microbes using the probiotic treatment. Thus, there is a place for adjunctive therapies such as probiotic treatment for enteric infections. Probiotics show the different mechanisms of probiosis within the host, such as prevention of pathogen proliferation, immunomodulation, mucosal barrier integrity, etc. More and more clinical trials need to be conducted to prove the efficacy and effectiveness of the probiotics and make them available at affordable prices. In this chapter, the role of different probiotics in the treatment of enteric infections, their mechanism of action, and clinical trials conducted are emphasized.

Keywords

Probiotics, clinical trials · *Escherichia coli* · *Clostridium difficile* · *Vibrio cholerae* · *Salmonella*

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12.1 Introduction

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 more considerable attention for newly demonstrated applications and effects (Pandey et al. 2015). The key to health and well-being lies in keeping a healthy gut.

The human gut (specifically the distal small intestine and colon) contributes to about 10^{13} diverse microbes (over 500 species) carrying approximately two million genes. This diverse microbial community is termed as our 'microbiota' and their gene pool together is called the 'microbiome'. The microbiota is responsible for carrying out different metabolic pathways generating an array of enzymes and metabolites that allow usage of different dietary compounds. Thus, microbiota, in due course of time, has earned its importance in the diet. Colonization of the gut with microflora begins in the infants during birth when the neonate is exposed to cervical and vaginal flora of the mother (Weizman et al. 2005). The foundation of a healthy gut is crucial in the first 2–3 years of life; this is the period where the brain-synapse development occurs, which in turn decides the human capacity.

Nutrients are required for any microbe to grow. The gastrointestinal tract (GIT) has an abundance of nutrients, thus apt for microbial colonization (McCormick and Lang 2016). The property of normal, healthy gut microbiota to generate a non-conducive environment for the growth of enteric pathogens is called colonization-resistance (CR) (Fuller 2012). Upon disturbance of the microbiota (due to stress, use of antibiotics, bad lifestyle, poor diet, etc.) CR is hindered, leading to either colonization by intrinsic pathogens or accelerated susceptibility to infections (Lessa et al. 2015). Infections trigger inflammatory host responses and pathogen-mediated disease(s) (Kelly and LaMont 1998).

Globally, there is a major burden of foodborne illnesses; every year, almost 1 in 10 people fall sick, and 33 million healthy lives are lost. In developing countries, enteric infections constitute a significant cause of morbidity and mortality. According to the Global Burden of Disease study 2016, it has been estimated that diarrhoeal illness causes approximately 1.7 million deaths annually and results in 74 million disability-adjusted life years lost. Table 12.1 enlists some of the common enteric pathogens, which are known to produce a toxin(s), causing fatal dehydration and diarrhoea by disrupting the intestinal functions (Britton and Versalovic 2008). The chronic exposure to infection-causing enteric pathogens such as *Vibrio cholerae*, *Clostridium difficile*, *Salmonella, E. coli,* and *Shigella* is associated with the stunting growth and developmental deficit in the children particularly living in low- and middle-income countries. An inadequate diet, which causes inflammatory reactions in the gut, altering the microbiome and metabolic and immunological pathways in the gut adds to the severity of the problem (McCormick and Lang 2016).

According to the Lancet Global Health 2018, the deaths due to *E. coli* and *Shigella* infections are increased by 24 and 28%, respectively (Troeger et al.

Table 12.1 List of common enteric pathogens

Sr. no.	Pathogen	Sr. no.	Pathogen
1	*Campylobacter jejuni*	8	*E. coli*
2	*C. difficile*	9	*V. cholera*
3	*Salmonella*	10	*Enterocolitica*
4	*Shigella*	11	*Rotavirus*
5	*Parahaemolyticus*	12	*Adenoviruses*
6	*Helicobacter pylori*	13	*Human caliciviruses*
7	*Novovirus*	14	*Astroviruses*

2019). The major transmission pathways for bacterial and protozoa enteric pathogens are via consumption of contaminated food and water and interaction with faecally contaminated environments, whereas viral by a human to human transmission (Knee et al. 2018). Though the diarrhoea or enteric infection is considered as an acute infection, it can cause long-term effect such as linear growth faltering, vaccine failure, cognitive deficits. These long-term effects are considered to be a large burden in years lived with disability. These pathogens change the gut integrity and impair the nutrient absorption, which in turn results in the malnutrition. This gave rise to an increase in infection and disease pervasiveness and weakened vaccine immunization. All of this plays a huge and inequitable social and economic burden on the families and the societies where they reside. An international project to reduce the morbidity and mortality due to enteric infections and malnutrition has been carried out where different intervention strategies are designed.

Susceptibility to infections by different microbes differs from one individual to another and is dependent on various parameters including genes like Interleukin (IL)-8 (Jiang et al. 2003), IL-10, IFNG, and Tumour Necrosis Factor-Alpha (Sicinschi et al. 2006); IL-1 (Pessi et al. 2005); IL-4/IL-13 (Thye et al. 2003); and Interferon Gamma Receptor 1 (IFNGR1) (Adedayo and Kirkpatrick 2008), IL-12B, IL-12RB1, and IFNGR1 (Dunstan et al. 2001), etc. Several pathophysiological studies have emphasized upon role of transport mechanisms and different signalling pathways as a result of which fluid and electrolyte transport in the small and large intestine is severely affected.

Antibiotic therapy is the most effective treatment for tackling enteric infections. However, its major concern is bacterial resistance to antibiotics. They perturb the natural microflora of gut and thus make hosts more prone to other invading pathogens such as *Salmonella,* etc. Microbiota perturbations during enteric infections are cyclical processes. Antigenic variability coupled with strain diversity adds further complexity (diagnosis or vaccine development or multiplicity of these agents and their serotypes) to deal with these enteric pathogens. As against the conventional antibiotic therapy, the need of the hour is alternative approaches to control enteric infectious in humans and animals (Paton et al. 2006). Probiotics have been reported to be potent in alleviating symptoms of enteric infections. The array of characteristics/parameters is taken into account (GRAS level organism, acid and bile tolerance, adherence, etc.) before labelling an organism as a probiotic (Pandey et al.

2015). Current scientific investigations are focusing on the relief in symptoms caused by enteric infections, malnutrition, etc., and more specifically exploring the potential of probiotics to selectively target viral and bacterial pathogens (Sleator and Hill 2007, 2008; Sleator 2010). Probiotics have also been explored for their role as vaccine carriers and have proved to have long-lasting protective responses (mucosal as well as systemic) (Czerkinsky and Holmgren 2010). This chapter focuses on some of the most notorious enteric pathogens and emphasizes the use of probiotics in treating the discussed enteric infections.

12.2 Most Notorious Enteric Pathogens

12.2.1 Vibrio Cholerae

Vibrio cholerae is a gram-negative, rod-shaped, bacterium, which usually spreads infections by the faecal-oral route. Consumption of food or water infected with *V. cholerae* causes enteric infection. The bacterium survives in the acidic (low pH) conditions in the stomach and later colonizes in the small intestine and secretes cholera toxins (CT) (Taylor et al. 1987; Mondiale and WHO 2017). As per the Sakazaki and Shimada typing scheme, there are 139 different O groups, of which *V. cholerae* O1 and O139 are the most (epidemic causing) pathogenic and dominating strains (Maheshwari et al. 2011). One can gauge the fright, *V. cholera* has created across the globe, by the number of institutions worldwide studying this disease—Naval Medical Research Unit (NAMRU), United States Army, Walter Reed Army Institute of Research (WRAIR), South East Asia Treaty Organisation (SEATO), Cholera research lab-Bangladesh, WHO-Diarrhoeal disease control program and the Centre for Disease Control, National Institute of Health (NIH), etc. (Giannella 1993; Mondiale and WHO 2017). Figure 12.1 depicts the *V. cholerae* pathogenesis (Rupnik et al. 2009; Clemens et al. 2011; Mondiale and WHO 2017). The pathogen survives the gastric acid barrier and penetrates the mucus lining that coats the intestinal epithelia. The bacteria adhere and colonize to grow and produce CT. It induces chloride and bicarbonate ion secretion, especially in the crypt cells of small intestine. Increased cAMP concentration in villi alters the intestinal functions such

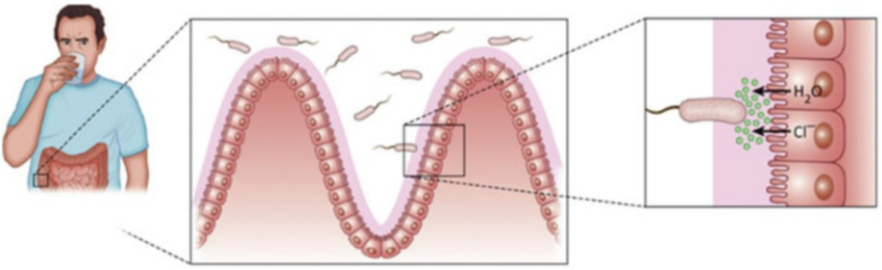

Fig. 12.1 *V. cholerae* pathogenesis (Adapted from Hun Yoon and Waters 2019)

Table 12.2 Vaccines available for some common enteric infections

Sr. No.	Disease/ pathogen	Vaccine/ (Manufacturer)	Sr. No.	Disease/ pathogen	Vaccine/(Manufacturer)
1	Typhoid	Typbar-TCV (Bharat Biotech)	5	Norovirus	Takeda vaccines
2	Rotavirus	Rotarix (GSK)	6	ETEC	Scandinavian Biopharma
3	Cholera	Shanchol (Shantha Biotechnics Ltd.)	7	Shigella	(LimmaTech/GSK)
4	Cholera	Euvichol (Eubiologics Co. Ltd.)	8	*E. coli*	Multivalent Bioconjugate (GlycoVaxyn)

that it starts working as a pump, which extracts water and electrolytes from blood and tissues and release into the intestinal lumen, causing the cholera symptoms (Maheshwari et al. 2011).

V. cholerae can switch between either of its 2 forms: motile or biofilm producing forms (Silva and Benitez 2016). *Cholera gravis* (severe form of cholera) results in profuse vomiting and watery diarrhoea, causing hypervolemia, in no time. Loss of water and electrolytes cause metabolic acidosis and potassium deficiency, worsening the condition further (Clemens et al. 2011; Reyes-Corcho et al. 2012). Vaccines come to rescue after the only option of antibiotic therapy. Some of the commercially available vaccines have been enlisted in Table 12.2.

12.2.2 Clostridium difficile

Clostridium difficile is an anaerobic, gram-positive, spore-former, which was first discovered from the faecal flora of healthy new-born infants (Kelly and LaMont 1998). *C. difficile* infections are endotoxin-mediated intestinal disease (Fig. 12.2) symptoms of which range from mild diarrhoea to fever, abdominal pain, and leucocytosis (Lessa et al. 2015). Most characterized endotoxins are toxins A and B, which results in diarrhoea and inflammation in infected patients, respectively (Di Bella et al. 2016). *C. difficile* persistently contaminates the hospital environment through spore formation. About 40% of *C. difficile* infections begin in nursing homes or community health-care settings.

Taurocholate and other bile salts in small intestine stimulate the germination of spores of *C. difficile,* thus the pathogen colonizes efficiently in the colon, especially where the normal microbiota is disturbed (e.g. by antibiotic therapy). Their adherence is enhanced by inducing microtubule protrusions that trap the *C. difficile* strains. Toxigenic strains produce toxin A and toxin B (TcdA and TcdB), which stimulates inflammation in colon. Disruption of tight junctions allows both TcdA and TcdB to cross the epithelium and further induce inflammatory cytokine production in lymphocytes and mast cells. These cytotoxic toxins induce release of immunomodulatory mediators form mast cells, phagocytes, and epithelial cells. It

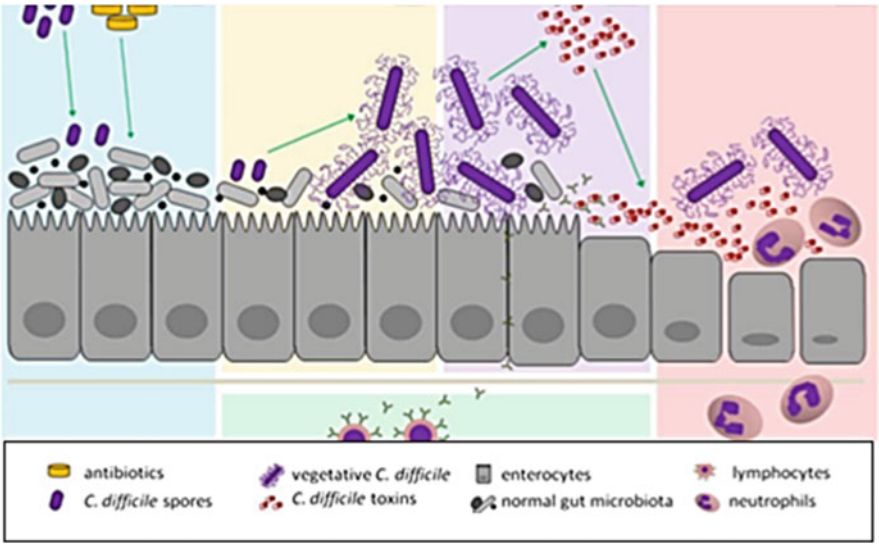

Fig. 12.2 Mechanism of *C. difficile* pathogenesis (Adapted from Fehér et al. 2017)

causes inflammation and the accumulation of neutrophils leading to pseudo membrane formation.

Poor sensitivity of methods (e.g. Stool based toxin assays, to detect infections) is the trickiest part of *C. difficile* diagnosis which prevents accurate diagnosis. Advanced molecular assays for real-time PCR detection of toxin genes directly in stool samples has significant diagnosis capability of antimicrobial associated diarrhoea and colitis, caused by toxigenic *C. difficile* (Britton and Versalovic 2008). The recent methods of typing *C. difficile* infections are toxinotyping, PCR ribotyping, restriction endonuclease analysis (rEa), amplified fragment length polymorphism (AFLP), multilocus variable number tandem repeat analysis (MIVA), multilocus sequence typing (MLST), etc.(Knetsch et al. 2013).

The curtailing of antibiotic use is critical for controlling the development of CDI; however, treatment for infections is inexorable in certain patients. The use of probiotics as an adjunctive therapy, in numerous cases, intervened the infections (Evans and Johnson 2015). Several bacteriocins have inhibitory effects on *C. difficile* infections like Nisin A, Nisin V, Thuricin CD, Lacticin, LFF577, Actagardine A (DAB), etc.(Rea et al. 2013). The patients (with history of recurring *C. difficile* infections) not responding to conventional antibiotic therapy are subjected to Faecal Microbiome Transplant (FMT) and have been beneficial in one procedure of FMT. Recent studies have demonstrated high success rates for restoration of normal microbiota by FMT, especially in patients with history of multiple *C. difficile* infections (Thompson et al. 2014).

12.2.3 Escherichia coli

Escherichia coli is a gram-negative, oxidase negative, motile/non-motile, rod-shaped bacterium from the family *Enterobacteriaceae*. It can be motile or non-motile, aerobic or anaerobic and has peritrichous flagella. Its isolation and characterization were first reported by Theodor Escherish in 1885 (Croxen et al. 2013). There are two major types of the *E. coli* serotypes which are based on Kauffman classification scheme. Total 227 serotypes, out of which 174 *E. coli* O (somatic) polysaccharide and 53 *E. coli* H surface antigens are determined (Nataro and Kaper 1998; Wang et al. 2003; DebRoy et al. 2011). According to Global Enteric Multi-Centre Study, *E. coli* and *Shigella* are two of the four major causative agents of moderate to severe diarrhoea among the children below age of 5, in Africa and South Asia (Levine et al. 2012; Anonymous 2012; Kotloff et al. 2013). There are almost 600,000 deaths in most of the underdeveloped countries due to enterotoxigenic *E. coli* and main cause of neonatal and post-weaning piglet's diarrhoea which becomes enormous economic burden to the swine industry (Qadri et al. 2005; Croxen et al. 2013). The major symptoms of *E. coli* infection are vomiting, diarrhoea, stomach cramps, loss of appetite, and mild dehydration (Qadri et al. 2005; Croxen et al. 2013).

The general mode of *E. coli* infections involves three steps—adherence, signal transduction, and intimate attachment. Depending on the type of *E. coli* strain, the mode of pathogenesis varies as shown in Table 12.3 (Clements et al. 2012). The intimate attachment of the pathogenic *E. coli* to intestinal cell induces different signal transduction pathways in the host cells which lead to subversion of different cellular processes for the benefit of the pathogen (Clements et al. 2012).

12.2.4 Salmonella

Salmonella is a motile, anaerobic facultative, non-sporulating, gram-negative straight rod belonging to the family *Enterobacteriaceae*. An American doctor, Dr. Daniel E. Salmon, had first isolated *Salmonella choleraesuis* from pigs with hot cholera in 1884 and the genus is named after him. Salmonella is one of the four causes of diarrheal illness, hospitalization, and deaths every year. In general, the Salmonella infection is generally characterized by fever, abdominal pain, diarrhoea, and sometimes vomiting leading to gut inflammation and sometimes lethal systemic infections. To date, more than 2500 serotypes or serovars have been identified, most of which are pathogenic. Some of them are host-specific whereas some have wide range of hosts (Anonymous 2018).

The major source of transmission is through the consumption of animal origin food such as eggs, meat, poultry, and milk as well as through green vegetables which are contaminated with manure. The person to person transmission can occur through faecal, oral route, and through the contact with infected animals. These infected animals generally don't show any symptoms. Almost 60–80% of all S*almonella* cases are not diagnosed or diagnosed as sporadic cases. *Salmonella* infection

Table 12.3 Types of *E. coli* and their pathogenesis

Sr. No.	Pathotype (Abbreviation)	Disease	Pathogenesis
1	Enteropathogenic *E. coli* (EPEC)	Profuse watery diarrhoea	– Localized adherence to host cells, – Signal transduction, – Intimate attachment – A/E lesion on host's intestinal epithelium
2	Shiga toxin producing *E. coli* (STEC)	Watery diarrhoea, haemorrhagic colitis	– A/E lesions on host epithelium surface – Different virulence factors such as Shigella toxin, Cytolethal distending toxin, EHE Chaemolysin, autotransporters
3	Enterotoxigenic *E. coli* (ETEC)	Persistent diarrhoea (<5 yrs); Traveller's diarrhoea	– Attachment to host's surface lining and then produces heat stable or heat labile enterotoxin
4	Enteroaggregative *E. coli* (EAEC)	Infant diarrhoea; Traveller's diarrhoea	– Biofilm formation on intestinal mucosa – Adheres to each other and to the surface and forms aggregative adherence pattern
5	Diffusely adherent *E. coli* (DAEC)	Acute watery diarrhoea (<5 year)	– Forms diffuse adherence pattern by dispersing over intestinal cells
6	Adherent invasive *E. coli* (AIEC)	Crohn's disease	– Invades epithelial cells and replicates within macrophages – Type I pili to adhere to intestinal cells and long polar fimbriae to invade
7	Enteroinvasive *E. coli* (EIEC)	Shigellosis; bacillary dysentery	– Intracellular pathogen – Penetrates intestinal epithelium through M cells – Inducts macrophage cell death and escapes submucosal macrophages

treatment generally considered of taking electrolytes to replenish the electrolyte balance lost during dehydration. To prevent *Salmonellae* infection, WHO recommendations for safe food are: keep clean; use safe water and raw materials; cook thoroughly; separate raw and cooked; keep food at safe temperature (Anonymous 2018).

Salmonella infects the host cells through macro-pinocytosis in which *Salmonella* injects an array of bacterial effector cell's molecules in the host cytoplasm and manipulates host cytoskeleton directly (Fig. 12.3). This results in membrane perturbing and forms bulky macropinosomes which contacts the engulfed bacteria. This method of infection is so vigorous that the inert particles and non-invasive bacteria get internalized with the internal bacteria and this happens within minutes of bacterial cell contact with host cell. Then they form the *Salmonella* induced vacuole where the internalized bacteria remained inside the membrane-bound vacuole. This vacuole is modified by bacteria to prevent its maturation or fusion with lysosome. Either of the 2 types of *Salmonella* infection can occur: gastroenteritis or systemic infection. In the latter type, *Salmonella* colonizes the organs like liver, spleen, and

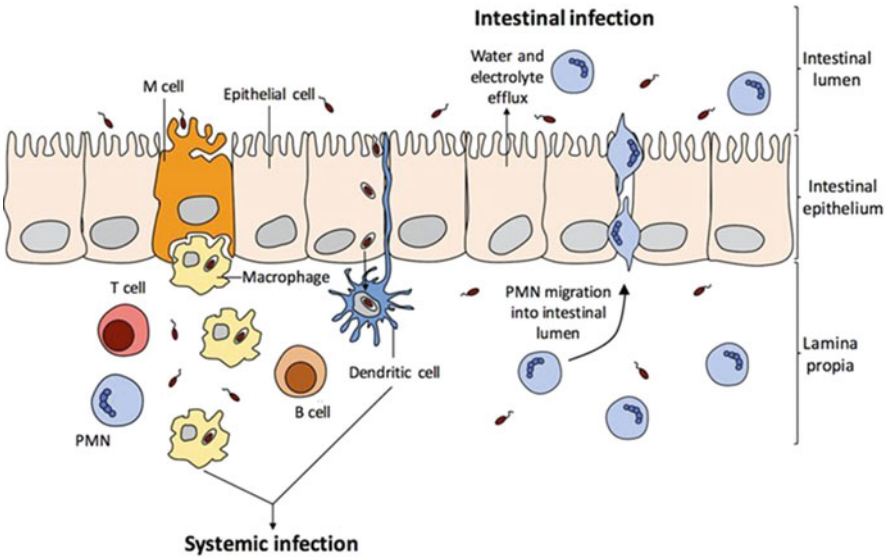

Fig. 12.3 Pathogenesis of *Salmonella* (Reproduced from Urdaneta and Casadesús 2017)

bone marrow before reaching the gallbladder where it results in chronic carriage (Urdaneta and Casadesús 2017).

12.3 Treatments of Enteric Infections

Different strategies have been applied to tackle the nuisance created by enteric pathogens, some include use of preventive drugs, use of vaccines, combining the use of antibiotics along with hygiene, and/or nutritional management, etc. Infections arising from enteric pathogens primarily demand use of antibiotics to effectively reduce the risk of severe health complications or death, in many cases. However, in the developed countries, unrestricted use of antibiotics has accelerated several cases of multidrug resistance amongst enteric pathogens like *Shigella* and *Vibrio* species. In developing or underdeveloped countries affordability and availability of these antibiotics is another major concern. Thus, the golden era of antibiotic discovery is towards its end and natural antimicrobials are getting increased attention for confronting this bacterial resistance. Another approach to combat the enteric infections is use of vaccines and literature cites several cases advocating the same. Pathogens like *Helicobacter pylori*, STEC*, V. cholerae,* etc. can be inhibited using vaccines. However, their efficacy varies based on disease burden. Recently, probiotics have gained an importance for their proven roles in prevention and treatment of enteric infections.

12.3.1 Probiotics

Probiotics were initially defined as living non-pathogenic organisms imparting health benefits on hosts. Since inception, the term probiotics has been defined and redefined several times. Pandey et al. (2015) give an account of modifications in the definitions of probiotics. Recently, FAO and WHO (2014) have jointly put forward the definition of probiotics as 'Live microorganisms that when administered in adequate amount confer a health benefit on the host'.

Several studies have reflected that many species of bacteria could be used as probiotics such as *Lactobacillus, Bifidobacterium, Pediococcus, Streptococcus, Bacillus and Escherichia coli*. It has been suggested that *Lactobacillus* and *Bacillus subtilis* have the paramount potential as probiotic therapies. Infants with chronic diarrhoea suffer from the stunted growth, low BMI, abnormal cognitive capabilities with adverse enduring health risks. Thus, the administration of probiotic to children is of great significance more than the gut health. Monachese et al. (2011) have proposed the systematic study design for the future study of the role of probiotic in the diarrhoeal diseases. According to the report, probiotics can be added for the reduction in the enteric and diarrhoeal diseases in global health strategies with proper selection of the targeted population. The primary responses should be reduction of diarrhoea episodes, lessened hospital visits or deaths and an overall public health improvement, with follow-up for at least a year. The secondary responses in case of infants could be impact on the height, weight, stunting, and cognitive defects. The highly dense population in the urban slum area of the developing countries could be the ideal choice for the targeted population.

12.3.2 Mechanisms of Action of Probiotics

Ng et al. (2009) have reviewed in detail the different mechanisms which probiotics adopt to bring about their benefits during enteric infections (Fig. 12.4). In general, the probiotics resist the enteric infections by antagonistic function, by enhancing antibody production or by limiting the access of nutrients by enteric pathogens, production of organic acids, etc. (Amalaradjou and Bhunia 2013).

Probiotics have been shown to produce antimicrobial compounds ranging from small organic acids to bioactive peptides called bacteriocins that act directly on the pathogens. Paneth cells (crypts of small intestine), intestinal epithelial cells, and other cell types in probiotic strains are known to produce antimicrobial peptides named defensins (Selsted and Ouellette 2005). They stimulate defensins production by producing proteases or MMPs. Volatile fatty acids are produced as a part of regular metabolism thereby reducing the pH of the GIT. Decreased pH makes the environment non-conducive for growth of pathogens (Mathipa and Thantsha 2017). Gut microbiota plays important role in the maintenance of epithelium barrier functions. In healthy individuals, the intact epithelium is crucial for effective nutrient uptake. Some reports advocate the upregulation of tight junction proteins through consumption of LAB. Additionally, LAB is known to inhibit the binding of

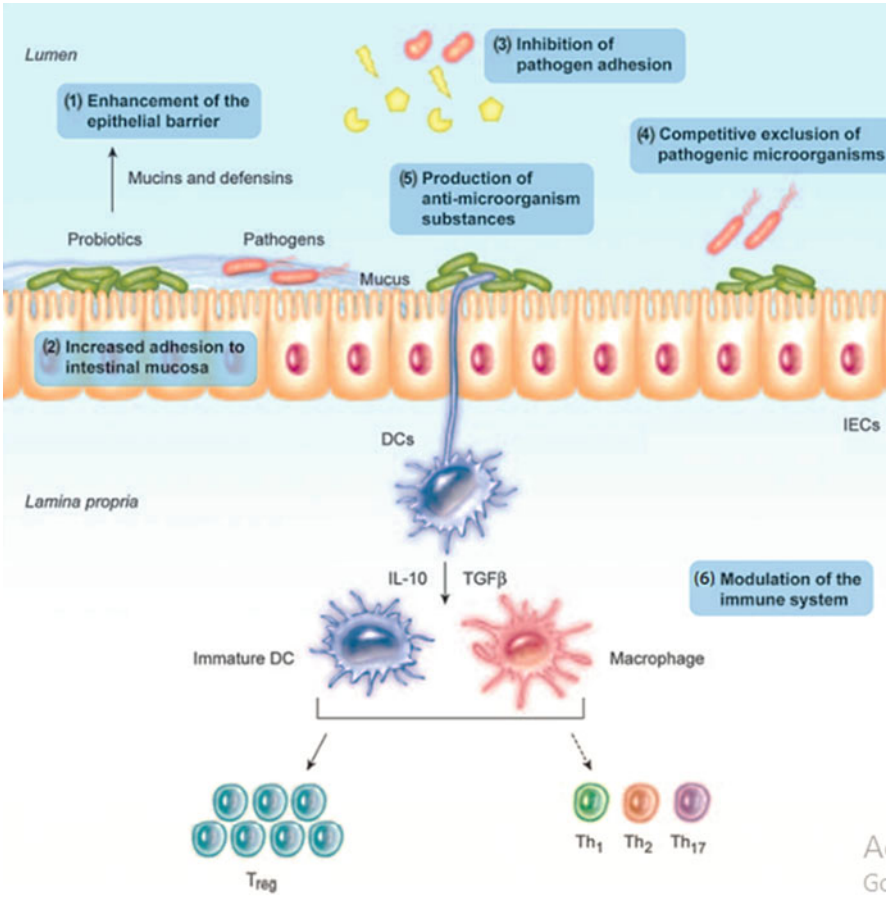

Fig. 12.4 Mechanisms of probiotic effects during enteric infections (Adapted from Bermudez-Brito et al. 2012)

pathogens to the gut lining coupled with downregulation of the toxin production by pathogens.

Probiotics employ mechanisms to adhere to the gut lining like specific surface proteins or forces like electrostatic communications (Ng et al. 2009). In doing so it leaves no space for pathogen binding. Competence of probiotics to adhere to the gut lining is higher than the gut pathogens (López and Urías-Silvas 2007). As they grow, they metabolize, one of the key metabolites, short chain fatty acids (SCFAs), produced by probiotic metabolic pathways. SCFAs are also known to decrease the cellular proliferation and induce apoptosis. The most abundant SCFA in colon is acetate, which can also be utilized by some butyrate producing bacteria in the gut.

Probiotics play important role in modulating host's innate and acquired immunity, as well, by improving IgA production, cytokine production (such as inflammatory, regulatory and interleukin-12 cytokines). Probiotics can interact with epithelial

cells as well as dendritic cells along with lymphocytes and monocytes/macrophages. IL production and NK cell activity also are modulated by these SCFAs by one of the mechanisms (Hijova and Chmelarova 2007; Jakobsdottir et al. 2013; Morrison and Preston 2016). Nuclear factor (NF)-κB coordinates all the immune and inflammatory responses against pathogens and other stress signals (O'Hara and Shanahan 2007). SCFAs not only reduce the pH in colon but also suppress inflammation and promote excretion of -ammonia and amine (Morrison and Preston 2016).

12.3.3 Probiotics Targeting Enteric Infections

Probiotics have the long history of safe consumption and/or contain the microbes that may colonize the healthy gut microbiota. Probiotic products contain different genera, different species, or even different strains of the same species, as probiotic activity is purely strain specific. There are two types of commercially available probiotic products, viz. monostrain (single strain) and multispecies (strains more than one genus). Most of the probiotic products do not make disease specific claim and therefore classified as food or dietary supplements. If the clinical data of study implies that it can be used for specific disease, then probiotics can fall under medical food. The probiotic is considered as pharmaceutical product if it makes any health claim that implies treatment, prevention, relief, or diagnosis of a disease. Thus, while formulating probiotic products, the following points are taken into account: probiotic microbe nature, mode of administration, dosage and also health condition of the patient, physiological function that probiotics are intended to perform (De Simone 2019).

In the last 10 years, probiotics have been studied, both in vitro and in vivo, for prevention and treatment of different enteric infections in humans and animals. The subjects ranged from mice, ducklings to pigs and humans. The in vitro studies were carried out in intestinal epithelial cell cultures like HT-29 and CaCO2. A summary of different recent studies of probiotics have been presented in Table 12.4, from which it is evident that probiotics do play a significant role in prevention, and in several cases, treatment of enteric infections.

To tackle the enteric infections multifaceted approaches have been adopted. The most common has been disruption of quorum-sensing system of *V. cholera,* as is demonstrated by *Ruminococcus obeum.* Designer probiotics have been developed (like *Lactococcus lactis*) that upon detecting quorum-sensing signals of *V. cholerae* enhance lactic acid production, thus decreasing the pH and making environment non-conducive for the pathogen (Chua et al. 2017). Engineered *E. coli* strains are available, which mimic the CT binding gangliosides on its surface, thus reducing the free toxins and curbing the infection. One of the approaches is to target the biofilm formation during enteric infections by pathogens like *V. cholerae* infection (Khailova et al. 2014). Different probiotic bacteria have been observed to inhibit the *S. enterica* infections by displaying certain surface properties like higher auto aggregation coupled with lower co-aggregation and hydrophobicity. The use of probiotics as an adjunctive therapy, in numerous cases, intervened the *C. difficile*

Table 12.4 Recent studies on treatment of enteric infections employing probiotics

Enteric pathogen	Probiotics	Clinical study	Major inferences	References
Clostridium				
C. difficile	*L. acidophilus* (CL1285); *L. casei* (LBC80R); *L. rhamnosus* (CLR2)	Single-centre, randomized, double-blind, placebo-controlled dose-ranging study/255 adults.	– Restoration of gut flora – Enhanced immune response to inhibit/destroy pathogen(s). – The probiotic load of this quantity (50 billion cfu) overcomes the intestinal flora and repopulates the gut. – Immunomodulation	Gao et al. (2010)
C. difficile	*Saccharomyces boulardii* and *Lactobacillus acidophilus*	5,574 hospital patients taking select broad spectrum antibiotics with probiotics versus without probiotics during the same encounter	– Statistically significant lower incidence of *C. difficile* infections	Hudson et al. (2019)
C. difficile	*Bifidobacterium breve* strain Yakult (BBG-01)	Randomized double-blind placebo-controlled study, Bangladeshi children <5-year-old/4 weeks with two doses of oral cholera vaccine	– Negative correlation between Bifidobacterium and Enterobacteriaceae	Matsuda et al. (2011)
C. difficile	*Lactobacillus* and *Bifidobacterium* strains	Randomized, double-blind, placebo-controlled trial of 17,480 patients >65 years and exposed to one or more antibiotics	– The multi-strain preparation was effective in prevention of AAD or CDD	Allen et al. (2013)
C. difficile	VSL#3	Multicentre, randomized, double-blind, placebo-controlled trial. 2 groups—placebo and probiotic, twice daily for the length of the antibiotics course and 7 days thereafter	– VSL#3 is associated with a significant reduction (18.6% vs 5.8%, $P = 0.02$) in the incidence of AAD in average-risk hospital inpatients exposed to systemic antibiotics	Selinger et al. (2013)

(continued)

Table 12.4 (continued)

Enteric pathogen	Probiotics	Clinical study	Major inferences	References
Vibrio				
V. cholera	*Bifidobacterium breve*	Randomized/128 children (2-5 years), 2 groups—placebo and probiotic. 2 doses of the oral cholera vaccine administered two-week interval following initiation of the probiotic/placebo administration.	– Immunomodulation – Can be used as adjunct to enhance the efficacy of the cholera vaccine in immunization programmes	Matsuda et al. (2011)
V. cholera	*S. cerevisiae* var. *boulardii*	100 subjects/*S. boulardii* (250 mg capsule) + 2 BS (Pepto-BismolH, P&G, Cincinnati, USA (chewable 262 mg tablets)	– Cannot be used as adjunct treatments – Boosts gastrointestinal immunity by decreasing inflammation – Inhibits few bacterial toxins	Sheele et al. (2015)
V. cholera	*Leuconostoc mesenteroides* (Lm) and *Bacillus subtilis natto* (Bs)	In-vitro	– Inhibitory effect on planktonic growth of *V. cholerae* – Biofilm forming ability – Competitive exclusion – Inhibited adherence to extracellular matrix proteins	VidyaLaxme et al. (2014)
V. cholera	*Lactococcus lactis*	In-vitro	– Reduced intestinal *V. cholerae* burden – Improved survival in infected infant mice – Acidification	Mao et al. (2018)
Salmonella				
S. typhimurium	*Kluyveromyces marxianus* S-2-05 and *Kluyveromyces lactis* S-3-05	In-vitro	– SopD gene downregulation in epithelial cell invasion – Acid and bile tolerant – Non-toxic for Caco2 cells – Repaired *Salmonella* induced epithelium cell barrier disruption	Ceugniez et al. (2017)

			– Ionic interaction and adhesion due to acidic mucin and strains (basic)	
S. enteritidis KCTC2021	*Lactobacillus plantarum* KCTC 3099 and *Sachharomyces cerevisae*	Broiler chickens: 240/0.4% DMLP and 0.4% DSMLP/7 days	– Antimicrobial activity by Chitosan in larvae – IgA and IgG levels increased – Dry mealworm larvae and super mealworm larvae probiotics used – Increase in average daily gain, average daily feed intake and decrease in feed conversion ratio	Islam and Yang (2017)
Salmonella	*Bacillus subtilis, Lactobacillus acidophilus, Pediococcus acidilactici, Pediococcus pentosaceus, and Saccharomyces pastorianus* mixture	Chicken: 64/106 cfu/kg feed/79 days	– Antimicrobial peptides – Combination of vaccine and probiotic is most useful	Redweik et al. (2020)
S. Typhimurium	*Clostridium butyricum*	Pigs/: 35/2 × 10^6 CFU·g^{-1} feed/ 42 days	– No change in faecal excretion, serological response, intestinal carriage and prevalence of *S. typhimurium* in the ileocecal lymph nodes – Dosage, duration, and time frame may have influenced the results	Peeters et al. (2019)
S. enterica subsp. *enterica* ATCC 35640	*Lactobacillus plantarum* DM 69	In-vitro study	– Antimicrobial activity – Inhibition of the growth, adherence and invasion of *S. enterica* – Reduced the biofilm forming ability of *S. enterica*	Mohanty et al. (2018)
S. Typhimurium SL1344	*Lactobacillus plantarum* ST-III	Mice/: −/5 × 10^8 CFU/18 days	– Improved intestinal function – Promotion of the Th1 response to deter systemic spread anti-inflammatory effects	Liu et al. (2019)

(continued)

Table 12.4 (continued)

Enteric pathogen	Probiotics	Clinical study	Major inferences	References
			– Inhibits the growth of Salmonella – Pretreatment antagonized all the defensive strategies of *Salmonella* – Improved the host resistance against pathogens	
S. Enteritidis	*Enterococcus faecium*	Broiler chicks: 24/−/11 days	– Supplementation of probiotic bacteria might therefore reduce energetic stress and improve muscle health and meat quality during SE infection	Zitnan et al. (2019)
E. coli				
Enterotoxigenic *E. coli*	*Lactobacillus rhamnosus* LGG and *Bifidobacterium* Bb-12	Mice: 80/1 × 10^8 CFU·kg^{-1} Bb12·day^{-1}/10 days	– Reduction in the inflammatory cytokine – Regulation of TLR4 MAPK signalling pathway – Combination of prebiotic and probiotic improved intestine barrier, inflammatory response and oxidant stress – Protection against ETEC induced intestinal damage	Tang et al. (2019)
Enterohemorrhagic *E. coli* O157:H7	*Bifidobacterium thermacidophilum*	Mice: 45/2 × 10^9 CFU·ml^{-1}/21 days	– Increased IgA – Mucin secretion to form a viscous gel that traps microorganisms and irritants and limit their access to epithelium – Pretreatment is recommended – Less Globlet cells infers less inflammation	Gagnon et al. (2006)

E. coli 17	*Lactobacillus casei*1.2435 *Lactobacillus rhamnosus*621 *Lactobacillus rhamnosus* A4	Ducks: 120/1.5 × 10^8 CFU·mL^{-1}/ 14 days	– Inhibits the growth of pathogens – Reduce colonization in gut flora, and promotes beneficial bacteria – Probiotics can modulate the gut microbiota in cherry valley ducks	Shi et al. (2020)
E. coli OP50	Cell free supplements of *Lactobacillus plantarum* K90	L4 stage nematodes of C. elegans	– Exclusion mechanism	Sharma et al. (2019)
E. coli KCTC 2571	Dry mealworm larvae Super mealworm larvae probiotics (*Lactobacillus plantarum* KCTC 3099 and *Sachharomyces cerevisae*)	Broiler chickens 240/3 different dietary treatments/ 0.4% DMLP and 0.4% DSMLP/ 1 week	– IgA and IgG levels increased – Antimicrobial activity by Chitosan in larvae – Increase in average daily gain, average daily feed intake and decrease in feed conversion ratio	Islam and Yang (2017)
Avian pathogenic *E. coli*	*Bacillus subtilis, Lactobacillus acidophilus, Pediococcus acidilactici, Pediococcuspentosaceus, and Saccharomyces pastorianus* mixture	64/106 cfu/kg feed/79 days	– Antimicrobial peptides – Combination of vaccine and probiotic	Redweik et al. (2020)
E. coli	*Lactobacillus plantarum* strain L15	In-vitro study	– Anti-adherence effects – Acid and bile tolerant – Inhibits adhesin of E. coli adhesion	Behbahani et al. (2019)

infections. Bacteriocins (like Nisin A, Nisin V, Thuricin CD, Lacticin, LFF577, Actagardine A (DAB), etc.) have inhibitory effects on *C. difficile* infections (Rea et al. 2013; Thompson et al. 2014; Evans and Johnson 2015).

Clinical studies to understand the implications of use of probiotics usage to treat *E. coli* derived enteric infections have been carried out in broilers, chickens, mice, nematodes, ducklings, ducks, and humans. Combinatorial method of probiotics with medicinal herbs and vaccines to treat *Shigella* infections has been encouraging (Redweik et al. 2020). Some plant-based therapeutic strategies have also been adopted, for example use of *Aegle marmelos* fruit lectin or the essential oil from *Cymbopogon martinii*, which has shown promising antimicrobial activity in vitro against pathogens like EIEC. Some probiotics (like *Lactobacillus rhamnosus*HN001 strain) have been incorporated in food products and non- pharmaceutical preparations for boosting immunity, specifically against *E. coli*O157:H7 infections (Shu and Gill 2002).

However, human clinical evidences of effectivity of probiotics in enteric infections need more efforts to correlate with the claims. This is demonstrated by the scarce number of completed human trials (27 out of 58), with the keyword 'enteric infections' listed on PubMed, and only 4 with the combination 'enteric infections probiotics'. Very few clinical studies (18) have been reported involving *C. difficile* pathogenesis—*C. difficile* infection (CDI), *C. difficile* enterocolitis, or *C. difficile* inducing diarrhoea whereas only two studies have been completed (Anonymous 2019). In case of *E. coli* infection, only two studies on *E. coli* induced gastroenteritis have been completed whereas there are no human clinical studies carried out on *Salmonella* enteric infections.

12.4 Concluding Remarks and Future Research Needs

In order to get control of pathogens, it is very necessary to understand in depth the pathophysiology, cross-talks between pathogens-probiotics and the epidemiology of enteric infections. Over the conventional antibiotic therapy, probiotics have demonstrated several mechanisms to counter the pathogens. Thus, probiotics play important role in the prevention and treatment of enteric infections. There is good scientific evidence that intervention by administration of probiotics bacteria may bolster colonization resistance. Probiotics show different mechanisms of probiosis within the host such as prevention of pathogen proliferation, immunomodulation, mucosal barrier integrity, etc. They can prevent the infection from occurring to suppress or diminish the severity and duration of the disease. Based on the human microbiome data, rational selection of probiotic as per the mechanism of disease and mechanism of probiosis can be facilitated based on the scientific evidences. Current regulations of probiotics are inadequate, especially when probiotics are used at dietary management of serious conditions. Stringent regulations addressing the medically beneficial probiotics which are not classified as drugs are required. Designer probiotics are going to be the new future in functional foods sector. More clinical studies need to be carried out to establish the efficacy of probiotics

and understand the mechanism of action. Also, further exploration is required to deduce better strategies involved in treatment of enteric infections. When combinatorial methodology with probiotics and multidimensional approach (comprising of improved standards of hygiene, sanitation, safety, breastfeeding of new-borns and through scheduled vaccinations, etc.) are coupled, the aim to end the endemic of enteric infections can be achieved successfully.

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Probiotics for Allergic Airway Infection and Inflammations

13

Satish V. Patil, Bhavana V. Mohite, and Vikas S. Patil

Abstract

Probiotics have expansively reported affecting the composition of the gut microbiota, and it opens promising areas of research for the discovery of probiotics in the prevention or treatment of infectious and inflammatory diseases. Probiotics exert multiple health effects such as immunomodulatory agents and activators of host defense pathways, influencing disease severity, and incidence. The normalization of the properties of unbalanced indigenous microflora by healthy gut microflora constitutes the rationale in probiotics therapy.

The probiotics microbiome is essential for the development of host immune responses, particularly within the context of allergy. The probiotics performance manifests itself in the normalization of the increased intestinal permeability, improvement of the intestine immunological barrier functions, and alleviation of the intestinal inflammatory response.

The effect of probiotics is based on the ability to differentially regulate the production of anti- and pro-inflammatory cytokines as well as the balance between types of T cell responses. Probiotics appear to be a feasible way to decrease the incidence of respiratory tract infections. Probiotics affect the lung

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immune response after the allergic airway inflammation due to an increase of T regulatory-dependent mechanisms. The proper development of bacterial colonization observed to downregulate the hypersensitivity reactions with alterations of the cytokine profile. There is a paucity of data regarding the study of the mechanism of probiotic. There is a need for a mechanism investigation of probiotic action to explore the putative benefit of respiratory disease.

Therefore, the current article focuses on the present scenario of the effect of probiotics on the immune system in allergic airway infections and inflammations.

Keywords

Probiotics · Gut microbiota · Allergy · Airway inflammation · Gut microflora

13.1 Introduction

13.1.1 Probiotics

The World Health Organization (WHO) defined probiotic as "living microorganisms in adequate amount confer the health benefits" (Food and Agriculture Organization of the United Nations, World Health Organization 2002). The phrase "probiotic" is a Greek term and means "for life." Originally it was termed as "substances secreted by one microorganism that stimulate the growth of another" (Lilly and Stillwell 1965). The redefinition by Parker (1974) coined the probiotics as "organisms and substances, which contribute to intestinal microbial balance."

The adapted narration by Fuller (1989) stated as "a live microbial feed supplement, which beneficially affects the host animal by improving its microbial balance." Marteau et al. (2001) provided the most accepted definition as "microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being."

The Food Safety Department, World Health Organization (2005) defined probiotics as "live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host." The international scientific community has admitted to this and has become the working definition of probiotics.

The most commonly used probiotics are *lactic acid bacteria* (LAB), particularly *Lactobacillus* and *Bifidobacterium* species, followed by the genera *Enterococcus*, *Streptococcus*, *Propionibacterium*, *Pediococcus*, *Escherichia coli,* and *Bacillus* (Szajewska et al. 2016). Few some yeast species are having potential as probiotics, e.g. *Saccharomyces cerevisiae* and *Saccharomyces boulardii* were utilized for the treatment of gastrointestinal diseases very often (Guarner et al. 2012; Sanders et al. 2013; Schreck Bird et al. 2017; Kerry et al. 2018). However, not all the bacteria can be probiotic, as they need to be strain-specific.

The probiotic produce is in the type of tablets, capsules, powders (which worked as a dietetic complement), and as a food component (e.g., kefirs, kombucha, tempeh, miso, yogurts, or a drug). The dairy products and functional foods are helpful for the restoration of healthy microbiota of the body and almost all adults, as well as

children, consumed it (Reid 2015). Hence dairy probiotic has been commercialized all over the world in different forms. However, allergy and lactose intolerance are the main arrests of dairy probiotics. The milk proteins, casein and whey proteins may act as allergens (Kumar et al. 2015).

Among the food factors, the use of food dyes is also a major reason for food allergy. Various natural and synthetic dyes such as carmine, tartrazine, and so on are added to the food to enhance the aesthetic value but may cause adverse reactions of food coloring allergy (Laura et al. 2019).

Probiotics are the indigenous nonpathogenic bacteria that colonize the mammalian intestinal tract. 10% out of 10^3–10^4 bacteria/ml dwelling in the body are legitimate living bacteria (Sender et al. 2016). The probiotic bacteria colonize initially maternal vaginal and fecal bacteria flora with reductive potential to make an anaerobic condition to favor the development of *Lactobacilli* and *Bifidobacteria*.

13.1.2 Benefits of Probiotics

The gastrointestinal tract is one of the most microbiologically dynamic environments that assume a vital role in the working of the mucosal immune system (MIS). The consumed probiotic stimulates the immune response as well as signaling by intact bacteria or its cell wall structure (Galdeano et al. 2019).

The gut is the site where huge numbers of bacteria from the microbiota and from the intestine which get through food intake coexist with each other. The immune cells are associated with the lamina propria of the villi. This intestinal microbiota does not interrelate straightforwardly with the epithelial cells; however, the maturation and functionality of the immune cells are stimulated by this microbiota through their metabolites (Hooper et al. 2012).

The beneficial effects of probiotics have been widely used in improving the host well-being and for the treatment of diverse infectious and non-infectious pathologies in animal models. Specifically it is included: protection from infection (Park et al. 2017; Acurcio et al. 2017; Mallina et al. 2018), irritable bowel symptoms relief (Hungin et al. 2013), reduction in the gut inflammatory response (Fábrega et al. 2017), cancer prevention (Aragón et al. 2015; So et al. 2017), growth inhibition of *Helicobacter pylori* (Fujimura et al. 2012), and allergies prevention (Velez et al. 2015).

Even though probiotics have shown encouraging results in several health conditions in humans, such as diabetes, multi-drug resistant pathogens, irritable bowel syndrome (He et al. 2017; Abdelhamid et al. 2018; Majeed et al. 2018), extensive research is still essential to include probiotics into human health, nutrition, and regulation of diverse abnormalities.

13.1.3 How Probiotic Function for Immune System?

The primary clause for probiotic microbes is survival in the harsh conditions of the gastrointestinal (GI) tract and stomach of humans. There are various ways by which probiotic microbes modulate the immune system. Figure 13.1 presents a brief of the role of probiotics for the immune system to maintain the human health majorly include: i) Modulation of innate and adaptive immunity, ii) Growth inhibition of pathogenic bacteria, iii) Regulation of anti-inflammatory or pro-inflammatory cytokines, iv) Regulation of the gastrointestinal /mucosal immune system (Baldassarre et al. 2016).

The important properties of probiotics which help to maintain the body to exert the effects are capacity to stick to the epithelial cells, activation of innate and cytokine-mediated immune response by internalization of a fragment of probiotic bacteria inside the immune response stimulating, intestinal epithelial cells (IECs) (Galdeano and Perdigon 2004), strengthening of the intestinal barrier by increasing the number of Goblet cells which reinforce the mucus layer (De Moreno de LeBlanc et al. 2008).

Table 13.1 summarizes the diverse means to promote human health. In recent years, extensive research has been conducted on the role of probiotics in transforming the adaptive and innate immunity as a way to check or treat a wide variety of health conditions (Baldassarre et al. 2016).

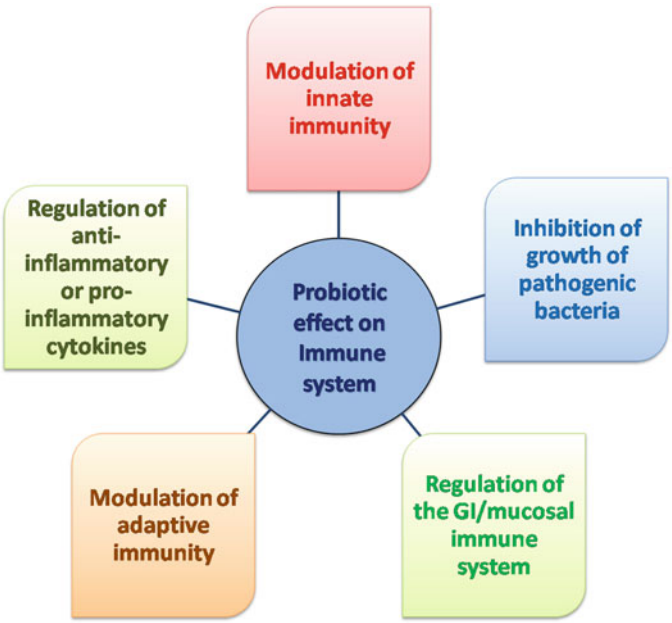

Fig. 13.1 Effect of probiotic on immune system

Table 13.1 Summary of probiotic mechanisms to promote the human health

Sr. No.	Mechanism	Active component	Reference
1.	Inhibiting the growth of pathogenic bacteria through the synthesis of inhibitory compounds such as organic acid, bacteriocins, antimicrobial peptides [29].	Acetic acid, lactic acid lactacin B, plantaricin lysozyme, secretory phospholipase A2, defensins, cathelicidins	Bermudez-Brito et al. (2012); Russell and Diez-Gonzalez (1997); Nielsen et al. (2010); Sankaran-Walters et al. (2017)
2.	Reinforce intestinal barrier integrity in tight junction signaling by amplified gene impression	Actin, zonula occludens-1 (ZO-1), actinin, occludin	Resta-Lenert and Barrett (2003)
3.	Protection of epithelial barrier and increased the tight junction protein expression with activation of signaling pathway	p38 mitogen activated protein kinases (p38 MAPK) and extracellular signal regulated kinase (ERK)	Dai et al. (2012)
4.	Increase in Paneth cells, produce anti-inflammatory metabolites,	Regulatory T cells (Treg) / type 1 regulatory T (Tr1) cells	Liu et al. (2016)
5.	Activation of adaptive immune system	CD4+ regulatory T (Treg) cells, dendritic cells	De Moreno de LeBlanc et al. (2005)
6.	Induction of different cytokines.	Interferon gamma (IFN-γ), tumor necrosis factor-α TNF-α	Jiang et al. (2013)
7.	Increases the phagocytic and microbicidal activity of macrophages	Specific antibody production	Núnez et al. (2013)
8.	Decrease of IgE	Immunoglobulin (Ig) G, interleukin 10 (IL-10) and IFN-γ	Fu et al. (2017); Jerzynska et al. (2016)
9.	Improving lipid profiles, reduce blood glucose and insulin levels	High-density lipoprotein (HDL)-cholesterol	Shah and Swami (2017)
10.	Anti-cancer effect by combination of multiple mechanisms	Anti-genotoxic and anti-gene mutation function, enzyme inhibition	Russo et al. (2014)

Now the probiotics have been commonly considered at therapeutical and clinical research level considering the relationship between the gut microbiome and immune disorders (Kothari et al. 2019), but the clear guidelines for the clinical application have yet to be established. This is particularly significant as the efficiency of probiotic supplementation may be reliant on the strain, dosing, condition, and duration of therapy (Toscano et al. 2017).

13.2 Role of Probiotics in Allergic Airway Infection

The normal healthy microflora constitutes the basis of probiotic therapy. Probiotics commonly mentioned as "good bacteria" or like a replacement for inhabitant stomach bacteria. Although the WHO recognizes probiotics as live microbes, when consumed in adequate quantity as an ingredient of food, it provides a health benefit to the host (Food Safety Department, World Health Organization 2005). At present, any item containing probiotics is viewed as a dietetic complement and is controlled by the principles and guidelines of the Dietary Supplement Health and Education Act of 1994. As indicated by it, the producer can give just common health declare for the manufactured food however it cannot express that any of the element in the product can fix, treat, or avoid illness (Alvarez-Olmos and Oberhelman 2001).

The dysbiosis, an inequity of the microflora constitution has adversely affected the health status. Three subcategory of dysbiosis have been recognized as below: (1) beneficial microbial agents loss, (2) spreading out of potentially harmful microorganisms, and (3) overall microbial diversity loss (Petersen and Round 2014).

Microbial dysbiosis has been concerned for different chronic inflammatory diseases, together with asthma (Sutherland and Martin 2007; Smits et al. 2016), chronic rhinosinusitis (CRS) (Hoggard et al. 2017; Aurora et al. 2013), Crohn's disease (Marin et al. 1983), and ulcerative colitis (Schmitz et al. 1999). The allergic infants reported an augmented number of *Clostridia* and a lower number of *Bifidobacteria* (Goktepe et al. 2005).

Amazingly all these persistent infections found to have altered membrane permeability and distorted functioning of epithelial barrier (Soyka et al. 2012; Steelant et al. 2016).

Probiotics have been publicized for a range of situation such as allergies, respiratory infections, including acute diarrhea, inflammatory bowel disease, and irritable bowel syndrome. This is been a choice to re-establish a healthy immune system (Dorval, 2015). Diverse probiotic strains and the mixing of microorganisms have a wide and differing range of clinical and immunologic potential and can manipulate gut microbiota in human beneficial ways (Table 13.2). The improved presence of probiotic bacteria in the intestinal microbiota has been found to correspond with defense from atopy (Moura et al. 2019). The predominance of hypersensitive ailment allergic diseases such as asthma, atopic dermatitis, and allergic rhinitis has expanded harshly over the past 2–3 decades in numerous nation, and sensitivities/allergies are presently most widely recognized chronic disease among youngsters all through the world (Tang et al. 2015).

The utilization of probiotic live forms could offer advantage to the patient's immunity, prompting improved management of the ailment, along with advanced lung functioning and reduced symptoms. Moreover, another mechanism of working of the probiotics comprised the enhancement in the epithelium membrane obstruction, hindrance of the adhesion of pathogens, binding to the intestinal mucosa, prohibition from pathogenic microorganisms by rivalry, and antimicrobial substance production (Bermudez-Brito et al. 2012).

Table 13.2 Representative studies demonstrating Probiotic effect in allergy

Sr. No.	Strain	Mechanism	Outcome	Reference
1.	*L. plantarum*, *L. lactis*, *L. casei*, *Lactobacillus rhamnosus* GG	Lesser IL-4 and IL-5 discharge	Reduced Th2 responses	Pochard et al. (2002)
2.	*Lactobacillus rhamnosus* GG and *L. bulgaricus*	Induction of IL1b, IL-6, IL-8, and TNF-a	Reduced Th2 responses	Niers et al. (2005)
3.	Lactic acid bacteria	Augmented IFN-g, TNF-a with IL-10	Reduced Th2 responses	Miettinen et al. (1998)
4.	*Lactobacillus rhamnosus* GG and *B. lactis* Bb12	Inducing transforming growth factor-β (TGF- β) secreting Tregs	Suppressed allergic symptoms	Feleszko et al. (2007)
5.	*L. acidophilus* W55	Stimulate functional FoxP3p(C) post-translational modification and Treg from CD25 cells	Supporting the species-specific effects of probiotics	de Roock et al. (2010)
6.	*Microbiota including* Bifidobacteria, lactobacilli,	Induction of mucosal IgA amount in addition to allergic B and T cell immunity	Modulation of allergy	Prescott and Björkstén (2007), Marschan et al. (2008), Galdeano et al. (2011)
7.	*Lactobacillus reuteri*	Reduced airway eosinophils, aryl hydrocarbon receptor (AHR) and TNF-a, IL-5 and IL-13 levels	Attenuate allergic airway disease	Forsythe et al. (2007)
8.	Commensal bacteria	Activation of DC andTh1 response	Stimulation of Th1 cytokines and, suppress Th2 response	Winkler et al. (2007)
9.	Commensal bacteria	Stimulation of mucosal IgA level	Allergen specific B and T cell response	Toh et al. (2012)

Allergic ailment represents a convincing challenge for community well-being concern due to their expanding predominance in evolved and evolving nations. Universally roughly 1 thousand million people are facing allergic symptoms and could be reached to 4 thousand million in the following 3–4 decades (Spacova et al. 2018).

Allergy is defined as a hypersensitive reaction to a particular antigen called an allergen by an immunological reaction (Ring, 2014). The commonly found allergies are against pollen grains, animal dander, mites of dust, or specific foodstuffs. Allergies are caused due to an increase in the amount of IgE (Akdis and Agache,

2014). The repeated exposure to allergen elicits activation of mast cell and basophile cells and release of allergic mediators like histamine and leukotriene resulting in five cardinal signs of allergy that vary from mild symptoms like sneezing but may become serious like difficulty in breathing and hypersensitivity.

The number of studies carried out to study the probiotic as therapy for airway allergy such as a Stockert et al. (2007) in a pilot study investigated the influence of probiotics for asthma suffering kids and discovered improved lung functioning (peak of expiratory flow [PEF]) but no effect on the quality of lives and use of asthma treatment. Furthermore, Chen et al. (2010) observed progress in signs, lung functioning, and immunological criterion in probiotic taking kids. Liu et al. (2016) described the effect of probiotics to improve the curative impact of allergen-definite immune treatment in asthma sufferers. The in vivo trial in rats having airways allergic inflammation when inoculated with *Lactobacillus reuteri*, improvement of inflammation and airway over sensitiveness in the probiotic receiving group of animals was observed (Forsythe et al. 2007; Karimi et al. 2009).

Moura et al. (2019) confirmed the role of probiotics as a complementary therapy for asthmatic children and teenagers. Furthermore, study is suggested to confirm the effectiveness of probiotics in asthma medication, particularly indiscriminate restricted experimental groundwork and ultimate cluster investigation, to assemble supplementary evidence and information on the promising expected advantage of probiotics for asthma sufferers.

There is a growing indication to put forward that each probiotic strain does not have a single exclusive mechanics of activity regardless of common taxonomical rank (Sanders et al. 2018).

The substantial cluster of proof is demonstrating that probiotics amend the type 1 helper T cell (Th1)/ type 2 helper T cell (Th2) (Th1/Th2) parity to forestall the improvement of inflammation infections such as allergy. The gut microbiota is having a vital role in re-establishing Th1/Th2 immunity.

The altered Th2 phenotype prompts an elevated number of IgE and hence activation of a mast cell, which will result in sensitivity to hypersensitivity disorders. The Th2- dominant phenotype of newborn displays higher receptiveness to hypersensitivity diseases. Amazingly, commensal colonization is contributed to this attribute, showing the important function of gut microflora. Commensals likewise assume a job in managing immune cell allocation. Therefore, susceptibility was accounted in adults following intense antibiotic course (Walker and Iyengar, 2015).

Another point of view of the perceptions is demonstrated in the "hygiene hypothesis." This recommends less microbial contact through early stages due to the improved community cleanliness. It is one of the essential reasons for uplifted receptiveness to allergic hypersensitivity. Likewise, these studies set up the role of microflora to affect the allergy immune response (Sharma and Im, 2018).

13.3 The Rationale behind the Mechanism of Probiotics for Allergy

This new strategy is originated from diversified information revealing the pleiotropic impacts of probiotics that incorporate immunomodulation, re-establishment of intestinal imbalance of microbiota just as keeping up epithelium hindrance solidarity (Toh et al. 2012).

Inflammation is an elementary defense mechanism of the immune system against unknown immunogen; however, allergy is a host defensive immunity on recurring presentation to a particular unknown particle as an antigen, yet possibly harmful to the horde. Inflammation is a type of innate immune response against the foreign virulent particles associated with tissue rejuvenation. Probiotics presumably work as immunomodulators and actuator of human defense mechanism, that propose to impact disease seriousness and its rate. Probiotics therapy is established on the idea of typical fine microflora. The probiotic therapy is based on normalization of the properties of unbalanced indigenous microflora by specific strains of the healthy gut microflora. The advancement of mucosal and fundamental resilience depends on immunosuppressant action coordinated by T cells that assuage both Th1 and Th2 responses, mechanisms may incorporate regulation of the useful properties of the microbiota, epithelial cells, DC, and safe cell types.

The superior adhesion properties of probiotic facilitate the maintenance of the mucosal barrier and avoid the absorption of foreign particles and expansion of IgA mediated immune response. The proper development of bacterial colonization observed to downregulate the hypersensitivity reactions with alterations of the cytokine profile.

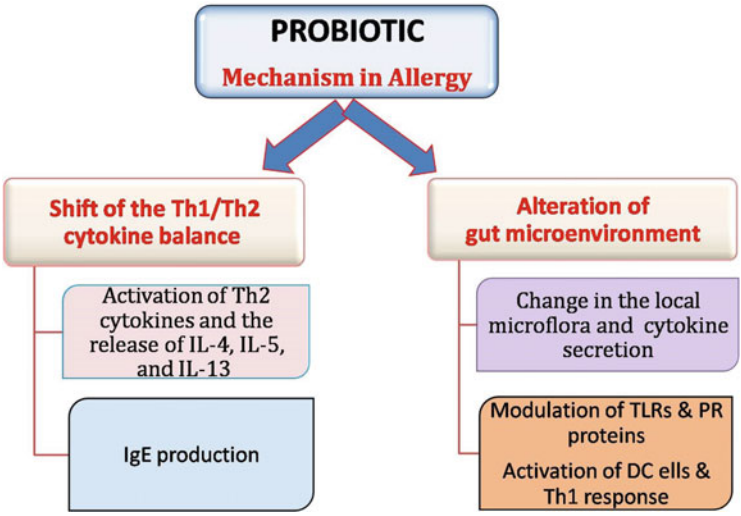

Fig. 13.2 Mechanism of probiotic in allergic reaction

Figure 13.2 describes the foremost activities of probiotic to undertake the airway allergic condition. The probiotic presents in the standardization of the extended intestinal permeableness and distorted gut microbial bionomics, development of the intestinal immunological fence job, and improvement of the response of gut inflammation.

The microbiome is fundamental for the advancement and learning of host immunity, mainly in the framework of allergic diseases. The use of probiotic influences the lung immunity followed by allergic airway infection due to augmentation of T regulatory-dependent mechanisms, however; whether this will impact the lung microbiota ruins to be determined. In reality, there is a need of elucidation of the mechanism of working of probiotic with assumed advantage for respiratory infections but there is paucity of data for airway microbiome composition.

13.3.1 Host Factors

The pathophysiology of susceptible illness, i. e. allergic disease results from an intricate series of actions including various ways of the natural immune response of innate and adaptive type. The allergic immune response involves stimulation of mast and basophil cells by IgE and succeeding allergen exposure resulted in allergic inflammation.

Host-associated factors can impact the working of the operation of the immune response in allergic hypersensitivity conditions and host and microorganisms communication (Laukens et al. 2016). Some vital characters are age, sex, host genetic structure, and microbiological status and can deviate in both human and animal investigation system (Laukens et al. 2016; Martín et al. 2017).

The pathogenic biofilm formation is the major host factor that leads to chronic infections. Biofilm formation is an accounted for about 65% and 80% of all microbial and chronic infections, respectively. Probiotic has the benefit as less cytotoxic than another quorum sensing (QS) suppressing agents and do not create strong pressure for resistance development like antibiotics. Hence probiotic could be an ideal alternative as an anti-virulent agent (Barzegari et al. 2020).

Probiotic prevents QS, biofilm formation, co-aggregation, and the survival of biofilm pathogens by interfering with biofilm formation and its quality. This is accomplished by decreasing the pH, competing for the adhesion sites with pathogens, and production of various antimicrobial agents like bacteriocin, hydrogen peroxide, and organic acids (Vuotto et al. 2014).

13.4 Allergy Prevention Studies with Probiotics

Current studies on meta-analysis of probiotics indicated a direct helpful impact on preliminary eczema impediment (Cuello-Garcia et al. 2015; Zuccotti et al. 2015), particularly to subsequent nativity to maternal and child to whom probiotics are administered. The probiotic will reduce the frequency of allergic sensitization with

perinatal intercession, which is not at all the condition for pre- or postpartum cure only (Zhang et al. 2016). Nevertheless, the support of probiotic for the avoidance of allergic airway disease is rare. There is no noteworthy outcome on the breathless incident or asthma improvement (Azad et al. 2013).

Lactobacillus probiotics strain is found to modulate the pro-inflammatory cytokines such as TNF-α, IL- 6, IL 1–10, and IL 1β by activating the macrophage (Rocha-Ramírez et al. 2017).

Probiotic consumption could decrease the occurrence of respiratory tract infections. Aerosol delivery of probiotic diminishes tumor seeding in the lung and improves chemotherapy against exploratory metastases. Probiotic seems to defeat commensal microbes incited tolerance encouraging the maturation of resident antigen presenting cells.

The prevention or repairing of "leaky" epithelial barriers could serve for the pro-inflammatory response. The epithelium barrier is the primary defensive physical obstacle of the individual for the entry of detrimental particles like any pathogen, irritants, and allergic compounds (Koch and Nusrat, 2012).

Eventually, probiotics can influence the inflammatory response by contrasting the basis of pro-inflammatory motivation related with low-quality endotoxemia. Besides, probiotics and some of their emitted metabolic products can straightforwardly influence key pro-inflammatory pathways by acting as ligands for innate immune system receptors. Intercellular junctions, for instance, tight junctions (TJs), adherence junctions (AJs), and desmosomes contribute to the construction and continuation of the physical barrier.

The probiotics have an advantageous impact on epithelium barrier malfunction which is widely considered for the digestive tract. The example may include *Lactobacillus plantarum* MB452 which elevate the articulation of TJ-related genes by in vitro testing in well abdominal epithelial cells (IECs) (Anderson et al. 2010).

Related encouraging impacts were confirmed in case of probiotic strains such as *Lactobacillus rhamnosus* GG (Orlando et al., 2014), *L. plantarum* MB452 (Resta-Lenert and Barrett 2003), *Streptococcus thermophiles* ATCC19258, and the gram-negative probiotic strain *Escherichia coli* Nissle (Ukena et al., 2007; Zyrek et al. 2007) on abdominal epithelium barrier intactness and TJ expression. Moreover, certain Lactobacillus strains show the potential to elevate epithelium barrier integrity through the stabilization of AJs expression (Hummel et al. 2012).

In particular, to mention, the tested lactobacilli strain enhances the E-cadherin and b-catenin and diminishes the ample protein kinase C expression in T84 human abdominal epithelium cell line. Protein kinase C is the enzyme responsible for the disassembling of adherens junctions (AJs) (Hummel et al. 2012).

Several barrier-rebuilding characteristics of probiotics have also been verified in diverse in vivo models (Laval et al. 2015). There are at present scarce reports in the airways, relating the dictatorial characteristics of probiotics on the epithelium lining. The oral medication with *L. rhamnosus* CRL1505 could circumvent the polycytidylic acid [poly (I:C)]-induced improved permeable nature of the bronchoalveolar-capillarity barrier for in vivo experimentation, as find out by albumin levels in the lungs (Zelaya et al. 2014). This progress was associated to diminish

the activation and synthesis of pro-inflammatory cells and cytokines in the lungs (Zelaya et al. 2014). Alike results were reported by nasally managed *Lactococcus lactis* NZ9000, which could neutralize *S. pneumonia* prompted permeable nature of lung tissue (Medina et al. 2008).

The in vitro studies reported dose reliant augmentation in epithelium obstacle functioning and reduction in epithelium permeability by prompting Calu-3 lung epithelium cells with the artificial bacterial lipopeptide Pam3CysSK4. This is caused due to improved articulation of the TJ proteins claudin-1 and ZO -1 and a lessen articulation of occluding.

Even though asthma is customarily viewed as a Th2-type inflammatory situation, it has been perceived as a clinically varied illness. The microflora composition of the gut and respiratory system is related to asthma incidents, as indicated by several reports. But it is not yet satisfactorily explained how disturbance of microbiota influences sensitivity to allergic asthma. It is projected that some metabolites formed during the fermentation of dietary fibers like short-chain fatty acids (SCFAs) by commensal suppress allergic airway responses (Trompette et al. 2014).

The Th2 response in the lungs is suppressed by higher serum SCFA, mainly propionate amending DC progenitors by G-protein fixed receptor in reliant way in the bone marrow. Butyrate is the foremost potent immune regulatory metabolite among the SCFAs. Histone deacetylase (HDA) inhibition is the mechanism of action for the butyrate and propionate function, with improvement in the acetylating status of histone in the Foxp3 site (Furusawa et al. 2013; Arpaia et al. 2013) and inducing tolerogenic DCs to augment Treg generation(Arpaia et al. 2013).

The Clostridiaceae family bacteria *Lachnospiraceae* and *Ruminococcaceae* are too recognized for the synthesis of SCFAs by fermented dietary fibers in the colon and thus sustaining epithelial integrity and homeostasis. But how this will helpful for humans, it needs to be confirmed by clinical trials (Sharma and Im, 2018).

13.5 Recent Advances: Clinical and In Vivo Status

In recent years, several experimental studies have investigated the capability of probiotic bacteria to improve the virulent traits of hypersensitivity disorders.

The animate models can be utilized in support of the probiotic impact and their systems of activity. This is found unrealistic in humans inferable from obscure dangers and moral concerns. The impact of such components should take into account during the experimental preliminary plan. The information exploration will encourage the advancement of superior probiotic intercessions and reinforce the proof for probiotic application in the prevention and cure of human beings ailment.

The effect of the human being genotype has likewise been proposed to assume a vital function in the result of probiotic medications, incorporating these acted with regard to allergic diseases. Individual hereditary contrasts and inclination towards inflammatory diseases ought to be thought about while surveying the impacts of probiotics in a clinical setting. The age of an individual and the influence of their gut

microflora should take into consideration for the human being testing. All around elegant study and strong in vivo and in vitro investigation are thus essential to advance definite choice of probiotic species for anticipation and management of allergic illness (Spacova et al. 2018).

To date, in any case, a large portion of the study on probiotic has concentrated on the microflora only as opposed to the interaction between host and microbiota. Additionally, accessible information discards the significance of mycobiome and virome. The existing screening system is centered on the cytokine production efficiency and capability of microbes by using the cell lines or ex vivo isolated peripheral immune cells, even though they do not symbolize phenotypically to gut cells. It is a requirement to develop high-performance screening procedures to ensure the particularity and sufficiency of picked probiotics. The majority of the commercial probiotic preparations are a combination of different bacteria with distinct colony forming units (CFUs). The purpose is learning of the consumer about the period for the viability of a specific strain and number of bacteria in specific dose.

Consequently, experimental testing should be extended to incorporate distinct geological areas. Considering this, it is advantageous not to execute meta-analyses on shared records when diverse strains of bacteria were utilized since the impact can vacillate drastically between the strains. The use of probiotic strains ought not to be permitted except the security and effectors compounds of the probiotics are very well cleared (Sharma and Im, 2018).

13.6 Safety Considerations and Contraindications

Immunomodulatory action may rely upon strain-specific characters so ideal strain might be presented. Probiotics are viewed with a safe, rare short term side effect (Ciorba, 2012). Isolated instances of bacteremia or fungemia have been related to probiotics, though inhabitants information additionally shows that there is no across the board danger of these complications (Snydman, 2008). Microorganisms that are "generally regarded as safe" incorporate species of *Lactobacillus* and *Bifidobacterium* and definite yeast strains. Other bacteria, such as *Enterococcus* and *Streptococcus* strains, are not generally considered as safe, however they are utilized as probiotics (Snydman, 2008). Itself alert ought to be practiced in prescribing probiotics to these populaces. Studies examining probiotics are comparatively short in length, limiting the long term security information and the ability for the real unfavorable circumstance. To make the firm ends, an additional experimental trial examining the safety of probiotics must be led.

The inconsistent outcome may result from the contrast in the cogitation plan, readout, and patient understanding. One significant impediment for an absolute meta-analysis of probiotic studies is the implementation of diverse probiotic species and strains, mainly *Bifidobacterium* or *Lactobacillus* or combination of that (Zuccotti et al. 2015). The administered probiotic doses also change significantly among the study from 10^7 to 10^{10} or more (CFU)/day, and treatment duration may also vary from a while to quite a while (Zuccotti et al. 2015). Nonetheless, the

outcome can vary among experimental set up in any event, though utilizing a similar probiotic strain and a similar direction routine because of the hidden possible significance of host-associated parameters. Along these lines, clinical studies heterogeneousness stays a significant hindrance to the conceptualization of validation-based rules on probiotic execution in allergic hypersensitivity (Forsberg et al. 2016).

Probiotics are susceptible to environmental surroundings such as moisture, heat, light, and oxygen. Customers should take precaution for storing probiotic containing product and adhered to the guidelines shown on the item label. One specific impediment restraint is the inability to indicate probiotic bacterial used for the study, depiction study duplication troublesome. Furthermore, numerous consumer diet complement exclude the particular bacterial strain or dosage of a probiotic on the mark, which makes it difficult for the drug specialist to advocate a product, in any event when a lesson is properly directed to deliver viable outcomes. Albeit numerous experimental testings bolster the protected use of probiotics, more exploration is expected to decide the long lasting safety of these items.

13.7 Future Directions

In current circumstance where the ebb and flow proof was created from hardly any preliminaries with serious extent of heterogeneity, routine utilization of probiotics as an added substance on treatment in subjects with unfavorably susceptible aviation route ailments cannot be suggested.

But the probiotic consumption emerges as a practicable way to diminish the frequency of respiratory tract diseases. Probiotics can affect together innate and adaptive immunity. Knowledge-based strategies supported with experimental data can be applied for successful clinical trials such as selection of optimal probiotic strain, microbe-derived compounds, the duration of regimens, administration forms, doses, and long follow-up time, as well as identification of potential early biomarkers of treatment efficacy. Recently scientist from Ireland, UK, and the USA propose the microbiome, live biotherapeutic product as a predictor of COVID-19 outcomes, for targeted immunomodulation in COVID-19 infection like prevention of virus attachment on host cells as well for prevention or treatment such as use of specific *Lactobacillus* strain as immunostimulatory adjuvant for intranasal vaccination, genetically engineered antigen producing organism. Consequently probiotics has great scope for the allergic airway infections which needs to determine.

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Probiotics as Edible Vaccines

14

Sahil Verma and Uttam C. Banerjee

Abstract

An era where an immunization has become a mandate to safeguard the biological system from the pathogens, vaccines have proven out to be the boon. Vaccines are an embodiment of microorganisms in an enfeeble state, just adequate to trigger the host immune system. Administered orally or through parenteral routes (IM: Intramuscular, SC: Subcutaneous, ID: Intradermal), vaccines evoke a defensive response of the biological system, i.e. the active type immunity. However, discomfort and anaphylaxis reactions associated with parenteral route are shortcomings. Thus, to bestow a better patient compliance, oral vaccines play a very crucial rule. An effortless administration of oral vaccines from paediatric to geriatric age groups with an increased patient compliance has initiated a league among the researchers to unearth potentials of oral vaccines. There are attempts of plant-based vaccines whereby the potential of plants as antigenic/epitope protein bio-factories are harnessed. The plant associated post-translational modification problems halt to leverage the clinical potential. The potential of probiotics to get adapted to gene manipulations as well as their human gut relations renders them suitable oral vaccines. Probiotics being in association with the gut microbiota may serve as immunomodulators by eliciting an immune response. The

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gut-associated immune cells rise to the bait during epitope encounter. The cellular immune response gets provoked and triggers the humoral immune response by a series of regulatory pathways to generate specific antibodies. An upcoming era is to fully leverage the potential of probiotics as successful therapeutic agents, though a far flung exploration needs to be unveiled for various research impediments in rendering the probiotics as antigenic/epitope bio-factories.

Keywords

Edible vaccines · Probiotics · Antigen · Immunization · Formulation

14.1 Introduction

Vaccines have been proved to be a boon in providing immunomodulation against various pathogens. Enormous decline in some of the most scourge infectious diseases has been made possible with vaccines (Sautto et al. 2019; Schuerman 2019). The use of the parenteral vaccines for long time has been associated with reduced patient compliance. Emerging understandings into mechanisms of gut immunity facilitated the concept of mucosal immunization *esp.* through edible vaccines which provide immunization through the oral route of administration. Lining of the gut epithelium has wide array of specialized cells which provide mucosal immunity as a first line of defence. Antigen encounter with the M cells triggers a local as well as a systemic immune response. *Antigen presenting cells* (APCs) play a most important role in providing the immunity by presenting an antigen to the specific cellular subset of immune system. APCs targeting strategies are in prevalence for directing the antigen towards the APCs so as to elicit a specific immune response (Saxena and Bhardwaj 2018; Sun et al. 2018; Cruz et al. 2019). Probiotics are prokaryotic microorganisms which are generally regarded as safe (GRAS) for human consumption. These microorganisms possess a unique ability to stimulate immune system of our body (Oh and Van Pijkeren 2014). They can be proved as excellent agents with vaccine potential because of their dual role of antigen production/expression as well as delivery agents to the site of immune targets for, e.g. *Bacillus subtilis*, Lactic Acid Bacteria (LAB) and *Saccharomyces cerevisiae* (Rosales-Mendoza et al. 2016). Due to the ease of stable genetic manipulations, heterologous protein expression has been made possible in the bacterial systems. Moreover, differential antigenic expression, i.e. intracellular, extracellular or surface expression can be specifically engineered to design the probiotics as targeted vaccinating agents (Jiang et al. 2019). Vast numbers of antigens have been expressed in the various probiotic strains till date. Extensive research has been carried out on rendering probiotics as targeted mucosal vaccine agents which has proved their tremendous vaccinating potential. Many of them are under the clinical evaluations (LeCureux and Dean 2018). This fetches a hope of switching a traditional vaccine prototype towards edible vaccines. However, risk of cross contamination and regulations governing the use of these genetically modified organisms are some of the limitations which need to be addressed for getting the

efficient vaccinated products in the market. Further sub-categorization of probiotics on the basis of their usage, viz. food supplements, drugs, etc. renders a variability in regulatory governance of the probiotic products which also vary from country to country (Venugopalan et al. 2010).

14.2 Probiotics in Mucosal Immune Response

Maintaining intestinal homeostasis is the mainstay of the gut immunity and is provided by the complex immune structure comprising of: gut-associated lymphoid tissues (GALT) or Peyer's patches, secretory immunoglobulins IgA (sIgA), antimicrobial peptides (e.g. defensins), mucosal immune cells (T cells), commensal bacteria, and various inflammatory mediators like cytokines, chemokines, etc. (Vela Ramirez et al. 2017). An intestinal epithelium comprises a myriad of cells with assigned functions. Enterocytes, goblet cells, Paneth cells, tuft cells, enteroendocrine cells, M cells, etc., are the cellular subtypes that are associated with intestinal epithelium. Among these, M cells and goblet cells are the major players in the mucosal immunity. M cells are responsible for an antigen uptake/capture. These cells further transfer an antigen to underlying immune cells via transcytosis, phagocytosis, microvesicle shedding. Goblet cells on other hand are associated with antigenic presentation to the dendritic cells (Allaire et al. 2018). Dendritic cells play a central coordinating role in providing a cellular as well as a humoral immune response. They act as Antigen presenting cells (APCs) by capturing an antigenic subunit from the microorganisms in the gut epithelia. Further fate is decided by either T cell or B cell activation via direct exposure or through an indirect exposure in the mesenteric lymph nodes. Presentation of an antigen through MHC I & II complexes leads to T cell's subset proliferation which ultimately confers a cellular immune response. In other context, B cell activation leads to the generation of secretory antibodies preferably IgA which is responsible for producing a humoral immune response (Palucka et al. 2010; Owen et al. 2013). Differentiation of cellular subsets inside the mesenteric lymph nodes renders immune protection by the process of gut-homing in which an effective immune response is provided alongside the lining of the gut by the variety of cellular subsets like $CD4^+$ cells, macrophages, $FoxP3^+$ T cells, etc. (Tokuhara et al. 2019). Probiotics possess excellent ability to amend the immune response towards the pro-inflammatory responses. Triggering of the cellular immune response occurs in the gut epithelium which provides a first line of defence. Further processing of the antigen by the immune cell of the gut confers the humoral immune response too (Kuczkowska et al. 2019).

Probiotics are generally the prokaryotic microorganisms which when ingested in an appropriate amount, provide a range of health benefits to the host (World Health Organization; Food and Agriculture Organization of the United Nation 2006). Probiotics are in use from the centuries by the human beings particularly in the form of fermentative foods like bread, cheese, beverages, etc. It was in the year 1907, when the noble laureate Elie Metchnikoff first discovered that probiotics cause an immune stimulation. With advancement in the recombinant DNA and molecular

biology techniques in the 80s and 90s, the concept of engineering probiotics emerged and it was in the mid-90s when probiotics were first used as antigenic delivery vehicles. This further broadened the horizon of using probiotics as antigen producing/expressing as well as delivery vehicles. The last two decades have witnessed a breakthrough research in the field of probiotics as edible vaccines. Moreover, with an emergence of more apparent mechanisms of immune systems involved in the gut, it has become easier to specifically design the probiotics to the targeted sites. Probiotics act on the mucosal immune system which is the first line of defence. The mucosal immune response in turn further triggers the systemic immune response through an array of signalling molecules. Hence, probiotics can be used to confer both the mucosal and systemic immunity. Probiotics have also been shown to have fewer collateral side effects as compared to the systemic vaccine responses (Bermúdez-Humarán et al. 2011; Ranasinghe 2014). Probiotics may considerably act by surpassing pathogens, producing toxin molecules to pathogens, or preventing their binding to the gut epithelia (MacDonald and Bell 2010). Human beings are tolerant to probiotics because of their co-evolved symbiotic relationship that have occurred via daily dietary consumption of the beneficial microorganisms. It is only when the abrogation of the tolerance persists, the fate of immune response persists for the probiotics (Erickson and Hubbard 2000). With their acceptability of being edible, probiotics possess an excellent myriad of paediatric to geriatric patient compliance. Due to the absence of side effects associated with the probiotic based vaccines, people of any age can be administered with probiotics at any time of the day (Alimolaei et al. 2017). Pattern recognition receptors (PRRs) are the type of receptors expressed on various epithelial cells of the gut which are responsible for interaction with microbiota (pathogenic or commensal). An interaction occurs via microorganism-associated molecular patterns (MAMPs) which further facilitates phagocytosis (Demento et al. 2011). However, due to variable MAMPs in different immune species, variability in immune response occurs among different species (Baarlen et al. 2013). The importance of probiotics is depicted in case of pathophysiologies (e.g. inflammatory bowel disease) which occur due to the dysbiosis, i.e. the altered balance between commensal and pathogenic microbiota, which leads to an unbalance between immune activity and regulation (Strober 2013).

14.3 Concept of Edible Vaccines

Vaccines are epitope/antigens with an activity restrained to a manner such as to elicit a trigger to an immune system of the host without any harm. From the first vaccine concept being introduced by the Edward Jenner, tremendous improvement has been observed in the areas of vaccinology. The vaccination strategy has proved to be a boon in the medical history. Eradication of some of the deadly diseases of the world like smallpox has been achieved with the help of vaccines and thus vaccines open up the doors to a new era of mass immunization concept to render the population of the world immunized in episodes of epidemics or pandemics (Colbère-Garapin et al. 2007; Ramezanpour et al. 2016). Since inception, vaccines have been administered

in the form of parenterals (intramuscular, subcutaneous, intradermal). However, limitations are posed in the use of parenteral vaccines like anaphylactic immune responses, swelling at the site of administration, etc., which ultimately hinders the patient compliance. It was only when Jonas Salk unveiled the first oral poliomyelitis vaccine in the year 1955 which was declared safe and effective to be used as it was composed of killed pathogens that were able to generate immune response without any infection (Salk Institute for Biological Studies 2016). From there, the edible vaccine concept emanated which idealized the use of live or attenuated pathogens which when fed orally, renders the mucosal as well as systemic immunization through the immune components associated with the gut (Mercenier et al. 2000). However, reversal of the attenuated strains back to their pathogenic form is the major limitation of using attenuated pathogens as edible vaccines (Boersma et al. 2000). Inconsistencies like socioeconomic factors, nutritional status, host genetic factors, pre-exposure to microbes are some of the hinderances in the vaccine use which must be addressed for leveraging the potential of probiotics (Ferreira et al. 2010).

14.4 Probiotics as Edible Vaccines

Section 14.2 described mucosal (gut) mechanistic pathways being elicited by probiotic microorganisms renders them suitable candidates for vaccinating strategies. Their exemplary role in the gut immunity along with their profound safer usage has manifested their distinct utilization as edible vaccines. Moreover, their affinity with M cells further renders them targeted antigen delivery agents. Following are the advantages that render probiotics best suited for edible vaccines:

14.4.1 Advantages of Probiotics as Edible Vaccines

With edible vaccines being a mainstay in the current proceedings of vaccinology, probiotic microorganisms possess certain characteristics that make them stand apart for their potential as therapeutic vaccine vectors:

- The ease of administration with acceptability in every age group is the major advantage of edible vaccines. Moreover, no trained personnel are required for dose administration (O'Hagan 1998).
- Simple and non-commensal microorganisms without any colonization in the gut, thereby posing minimal side effects.
- LAB (Lactic acid bacteria) are preferable choice for non-invasive route of administration (i.e. oral or nasal) either for prophylactic or therapeutic purposes.
- The biological machinery of these microorganisms is easily susceptible to efficient genetic manipulation with prolonged stability,
- Therapeutic vaccines made using LAB are cost effective in comparison to the other vaccines. Moreover, daily dosing can be rendered possible with an ease by probiotic based edible vaccines.

- These microorganisms serve a dual role as vaccine bio-factories as well as oral delivery vehicles, whereby the antigenic expression inside the genome of the probiotic can be steered towards the specific site expression which can then be presented to the immune cells of the gut.
- These microorganisms are omnipresent, i.e. their ubiquitous nature facilitates their efficient usage throughout the world (Wells and Mercenier 2008; Rosales-Mendoza et al. 2016).
- Probiotic species of *Bacillus* owes an endospore forming property and these spores possess an excellent property to resist harsh environments. Therefore, these traits render them suitable vectors for edible vaccines either in dormant or vegetative forms (Duc and Cutting 2003; Amuguni and Tzipori 2012; Song et al. 2012).
- Site specificity can particularly be imparted into the probiotics which in turn pose produce heterologous antigens with an exposure on a specific targeted site in the gut epithelium (Singh et al. 2017).
- The genetically modified probiotics can also be used for the expression of various immune components like cytokines, interleukins, etc. The expression of these immune components further widens the vaccine potential in an array of pathophysiologies *esp.* in the various cancer prophylaxes (Zhang et al. 2008).
- In the search of an alternative strategy, probiotics have shown their effective potential for the prevention of the malaria. This further widens the scope of leveraging probiotics in combating some of the deadliest diseases of the world (Ngwa and Pradel 2015).
- Probiotics show promising strategies to combat viral infections. Most of the trials on animal focussed on viral antigenic expression in probiotic microorganisms have shown promising results. Thus, probiotic's potential can be leveraged for major viral outbreaks like HIV, herpesvirus, influenza virus, etc., (Cortes-Perez et al. 2007; Yang et al. 2017; Sun et al. 2018; Kuczkowska et al. 2019).

14.5 Industrial and Clinical Outlook

The far broaden horizon of edible vaccines provides a cost-effective insight *esp.* in case of mass vaccination strategies wherein the requirement for disposables (syringes, gloves, etc.) as well as the assistance by medical professionals for vaccine administration are ruled out. Stability of edible vaccines as well as lesser stringent norms for transportation (cold chain) too lowers down the hefty expenses as reckoned in case of parenteral vaccine products (Streatfield 2005; Criscuolo et al. 2019). Several factors govern the commercialization of technologies from the research to the industrial scale. Formulating an edible vaccine by manoeuvring the probiotic cellular machinery is a cumbersome task that requires a huge set of precision experimentation and engineering in carving out the specific role of the probiotic to the fullest and that too with minimal or nil adverse effect. Adherence to stringent norms for Good Manufacturing Practices (GMP) is a major factor which contributes to the successful instigation of the probiotic based vaccine product. The

manufacturer of the probiotic based vaccine product must adhere to the set of GMP rules and regulations as laid down (and amended in case of current Good Manufacturing Practices cGMP) by the competent authorities timely so as to ensure a safe and efficacious product. An evident fact also makes it obvious for the probiotic based vaccine products to fetch more stringent GMP requirements as compared to the probiotic based food products. Albeit satisfactorily, framework for probiotic production has starting emanating from different nations of the world, yet the coordinated framework at global level for GMP guidelines is far behind the track. Further detailed GMP scenario on probiotics has been described elsewhere, for which the readers are suggested to refer Arora and Baldi (2015). It takes years of research and experimentation with combined efforts of molecular biologists, formulation scientists, and clinical researchers to design a product and come up with a formulation of an edible vaccine (Fig. 14.1). Huge amount of research has been focused on the targeted vaccine designing as described below.

14.5.1 Edible Vaccines-Strategies of Production

With the advancement in vaccinology, the paradigm for edible vaccine started shifting towards other alternatives for antigenic production. The antigen production has been carried out in the various organisms like plants, algae, silkworm, yeasts, and Gram-positive bacterial species. Each of the organisms (Table 14.1) possesses one or the other traits to render them suitable for transgenic expression of antigens. However, with organism other than yeast and bacteria, deeper insights are needed for an exploratory research into the yield optimization of the protein expression. Moreover, getting knowhow of post-translational mechanism involved in these systems also need a validated analysis. Therefore, all these pitfalls associated with the above described organisms facilitate the usage of yeast or bacterial systems in the transgenic protein expression (Rosales-Mendoza et al. 2016).

14.5.2 Formulation Aspects in Designing Probiotics as Edible Vaccine

An edible vaccine with enhanced bioavailability and safer usage profiles is a formulation scientist's paradise. The purified antigens are generally labile to the various mucosal barriers which lead to a loss of antigenic activity. Use of probiotic microorganisms renders an advantage to the antigen as they are presented as particles to the immune system (Ensign et al. 2012; Leenhouts 2013; Singh et al. 2018).

Vectorization of the antigens plays an important role in the delivery of antigens via oral route. Liposomal as well nanoparticle delivery strategies can be used for the vectorization of the antigens being expressed by the designer probiotics. Vectorization may render an ideal probiotic edible vaccine wherein: (a) it is capable of withstanding the harsh acidic and basic conditions so as to protect the antigen of interest, (b) it may preferentially be present the antigen to the specialized cells

Fig. 14.1 Edible vaccines: from process to productivity

Table 14.1 Various organisms deployed for edible vaccine production

Vaccine producing organisms	Advantages	Disadvantages
Plants	Easy process scale-up; high capability of post-translating proteins	Excessive post-translational modification
Algae	No transformation needed; growth regulators are not desired; protein folding ability	Lower yield of protein; difficult process
Silkworm	Higher level protein expression; acid and bile resistant proteins because of protease inhibitors	Acceptability of being edible
Yeasts	Higher cell mass yield; easy handling; prior process knowledge	Hyper glycosylation
Bacteria (gram-positive)	Rapid process development; shorter growth cycles, hence, shorter production times	Post-translational modifications; regulatory requirements

(M cells) of the GALT, (c) it may have a balanced uptake of both the antigen and adjuvant to cause a specific immunity. Beyond that, sustained antigen release, possible enhancement in the retention of the antigen at the site and the modification of the antigen presentation to cause generate specific immune response are some of the other benefits which are associated with the antigen vectorization. Moreover, applying the concepts of Nanotechnology to the edible vaccines have rendered their more safer usage profiles as well as improved vaccine efficacy. This further opens an arena into the new field of research for enhancing the potential of edible vaccines by nano-delivery agents (Pavot et al. 2012; Barhate et al. 2014). However, even with the use of probiotics, the fate of an antigenic immune response is nevertheless assured until the formulation has been properly optimized for an appropriate immune response. Formulating an edible vaccine with probiotics involve a number of parameters that are affected by the properties of both the antigen as well as the microorganism. An ideal edible vaccine form created out of probiotics should possess the following properties:

Stability Rendering a stable formulation is a pharmacist's paradise. Being edible in nature, the vaccine formulations in the form of suspension or tablet or capsule are desired. Therefore, the foremost goal awaited is to render formulation stability in the gut for which pH stable formulations or coated formulations are required. In the past decades, number of coating materials on micro and nanoscale have been developed and validated which are desired for enhanced oral drug stability as well as efficacy (Govender et al. 2014). Due care is needed while formulating an antigen *esp.* in the case of proteinogenic antigens which are labile to the various manufacturing processes such as lyophilization, etc. Process parameters must be rendered optimized for vaccine to retain sufficient viability throughout its shelf life (Huyghebaert et al. 2005). The vaccine formulation must tolerate harsh conditions of the gut such as acidic pH, bile salts, etc. In case of protein antigens, the formulation must resist enzymatic degradation by the proteolytic enzymes in the gut (Seegers et al. 2005).

Live vs. Killed Microorganisms Vaccine efficacy is also dependent on the viability of microorganisms because de novo synthesis of an antigen is associated with the multiplication of live microorganisms inside the host. Hence, variable antigenic titre is associated with the use of live microorganisms as vectors (Wells and Mercenier 2008). Moreover, immune response should be targeted against an antigen instead of live microorganism. Ideal vaccine design with probiotics demands an immune tolerance towards microorganism instead of antigen. An ideal edible vaccine formulation should not generate autoimmunity or hypersensitivity (Wu and Weiner 2003; Rhee et al. 2012).

Killed microorganisms in contrast have been used as carrier systems for the delivery of purified antigens. Use of probiotics as carrier systems for the antigen delivery has further been enhanced by the modifications with technologies like *MimoPath™ systems* developed by Mucosis-company. It involves the concept of using non-living probiotic microorganisms (Gram-positive microorganisms only) as vectors for the antigen loading. These microbial vectors act as adjuvant as well as target delivery agents for the purified antigen. The technique involves the use of harsh acidic conditions to treat a Gram-positive microorganism which renders a destruction of the cellular components, leaving behind bacteria like particles (BLPs) which are then mixed with the purified antigen to render a BLP-antigen vaccine. Out of various Gram-positive microorganisms, *Lactococcus lactis* has been proved to be the most suitable microorganism for the above said purpose. Favouring a Th1-specific immune response by *Lactococcus lactis* further enhances its utility in the vaccines (Van Roosmalen et al. 2006; Leenhouts 2013). Hence, *MimoPath™ systems* offer unique strategies for the edible vaccine production because of the advantages associated with the techniques.

Antigenic Adhesion and Presentation An ideal edible vaccine must possess mucoadhesive properties. An antigen must bind to particular cell substrate (For e.g. M cells) on the epithelial lining of the gut and then captured and presented to the immune cell subsets (as described earlier). Thus, an edible vaccine formulation with mucoadhesive properties as well as a targeted antigenic presentation is desirable for an effective immune response. Further details of targeted vaccine designing are provided in the following section.

Prolonged Immune Response Due to lesser colonizing potential of commensal microorganisms inside the gut, transit time of antigens decrease when edible vaccines are administrated. New strategies for enhancing vaccine's transit time inside the gut must be developed. However, vaccination schedules and coordinated delivery of adjuvants and antigens need further proof of research for its validation. An ideal vaccine designing encompasses a coordinated antigen and adjuvant delivery along with their presentation to the immune cellular subsets so as to prevent their cross-presentation (Woodrow et al. 2012).

14.5.3 Probiotics as Targeted Oral Vaccines

With a specific knowhow of probiotics targeting the specific immune components, designer probiotics can be rendered for a targeted action. Specific immune sites on the intestine like Peyer's patches containing M cells and other sites of Gut-Associated Lymphoid Tissue (GALT) along with various receptors like Toll Like Receptors (TLRs) are the main vaccine targets. An appropriate balance between the tolerance (to commensal microorganisms) and the pathogenic immune response is regulated by the mucosal immune system. Mucosal adjuvants, vectors or delivery platforms are therefore required with various mucosal antigens to elicit a targeted immune response. Adjuvants are responsible for an immunomodulation. An ideal adjuvant elicits a specific immune response by activating the specific immune component in the intestine to an extent where a balance between the toxicity and adjuvanticity persists. With number of chemical adjuvants like aluminium salts in use, understandings in the mechanisms by which these microorganisms act as adjuvants have facilitated their use as vaccine adjuvants. However, care must be taken for a generation of specific immune response against a heterologous antigen instead of the whole microorganism (Guy and Burdin 2005; Bahey-El-Din 2012; Rhee et al. 2012; Woodrow et al. 2012).

With an absence of targeted response in the so-called classical delivery systems such as attenuated pathogens, microparticles or liposomes, lactic acid bacteria (LAB) represent an alternative in edible vaccine development for their targeted immune response potential (LeBlanc et al. 2013). LAB being non-pathogenic and non-invasive in nature is the most suitable candidates for the heterologous protein expression. Absence of the protease secretion further increases the possibility of the stable protein expression. Most of the heterologous protein expression studies in recombinant LAB have been carried out with NICE (Nissin Induced Controlled Expression) system. In contrast to intracellular or extracellular protein expression, surface expression is a preferable choice in case of oral vaccines.

DNA vaccines harness the role of entero-invasive microorganisms which invade the intestinal epithelium and render the DNA delivery to the APCs. With this technique, multitude of antigens can be expressed by the use of single DNA vector (Daudel et al. 2007). Recombinant LAB can also be used for DNA vaccine delivery wherein the post-translational modifications of the expressed antigen occur in the host cells. The conformationally restricted antigen thus produced is presented to the specific components of immune system thereby facilitating a targeted immune response. To render it more efficacious, invasive strategies in which a gene for a protein is expressed into lactic acid bacteria mediating the invasion. For example, *inlA* gene from *Listeria monocytogenes* encodes for the internalin A surface protein when expressed in LAB, promotes its' internalization into the human epithelial lining (Bermúdez-Humarán et al. 2011). Modified bacterial vectors (for e.g. with nanoparticles) have shown an enhanced efficacy of the DNA vaccines in the cancer treatment (Hu et al. 2015).

M cell targeting therapies are currently in vogue whereby, the specificity is rendered to the vaccines/antigens for their site-specific adhesion and antigen

presentation to the M cells. Specific molecules *esp.* the peptides having an affinity for the M cells are linked to the antigenic vaccines so that the antigen can be delivered to the targeted site (Singh et al. 2015). However, paucity of M cells in the gut epithelium arises a need of other targets for enhanced vaccine potential. In lieu of that, other receptors are being explored for targeted immune response. Number of lectins, pattern recognition receptors (PRRs), and Toll like receptors (TLRs) are currently under investigation due to their distinguished role in activation of innate immune signals (Demento et al. 2011; Barhate et al. 2014; Kim et al. 2019).

The most important application of the probiotics has been found in the prevention and treatment of several types of metabolic disorders, allergies, infectious diseases as well as cancers. Generally, vaccination strategies are meant for the prevention of the disease. However, designing probiotics in a programmable manner have rendered their use for the treatment of various metabolic disorders as well as tumours. The inducible circuits of the probiotics can be programmed in a manner so that whenever they encounter any quorum sensing signals from the nearby tumours or infected cells, they release certain factors against the tumour or infected cell. This finds a most important application in the pathophysiologies and cancer related to the gut. Further details of the programmable probiotics for their use in cancer are beyond the scope of this chapter and readers are suggested to refer the particular readings (Chua et al. 2017; Wagner and Ichim 2017). Other oral mucosal routes including buccal mucosa, gingival mucosa and sublingual mucosa are currently explored because of the enhanced mucosal and systemic immune responses being triggered through these routes of vaccine administration (Pavot et al. 2012). The sublingual route of administration bypasses the gut-associated mucosal barriers. The capacity of probiotics as adjuvants in sublingual vaccinating strategies may render a promising strategy for allergies humans (Van Overtvelt et al. 2010). Hence, targeted immune response is a variable of two factors, namely; *microbial diversification* wherein the specific probiotic species or strain has a potential to interact with specific immune component, thus rendering it possible to leverage the potential of that specific species or strain for targeted vaccine response, whereas in *multivariate interaction* of a same probiotic strain with the different types of immune components, the antigenic enrichment for a particular immune cell receptor can be continued in vaccine development by various molecular biology as well as genetic engineering techniques to confer a probiotic vaccine with a specific immune target. Several formulation aspects along with these two variables further enhance the targeted effectiveness of the vaccines.

14.6 Challenges Associated with Probiotics as Edible Vaccines

The advancements in the probiotic based vaccine technologies has reached to the clinical settings (LeCureux and Dean 2018). Although there are considerable perspectives promoting the research in an area, yet various hurdles are also being posed: they are as follows.

- Due to protein nature of antigens, edible vaccines are amenable to physic-chemical (pH, bile salts, proteolytic enzymes, etc.) and biological (intestinal epithelium) barriers in the gut. Designing an appropriate delivery system with efficacy and safety demands an extensive research in the formulation and development which must overcome the associated barriers with oral drug delivery (Ensign et al. 2012).
- Selection of a suitable strain for the heterologous protein expression is a challenge because of the genetic diversity associated with the probiotic strains particularly with LAB. Different strains have different types of an expression and therefore it often becomes cumbersome task to select an appropriate strain for an antigenic expression (Mota et al. 2006; MacDonald and Bell 2010).
- Plasmid based system poses a diminished expression of the proteins. Also, the horizontal transfer of the recombinant and antibiotic resistance genes of the modified probiotic microorganisms is a major risk being posed to the environment. Therefore, proper regulatory guidelines are desired for the safer use of these microorganisms in the clinical settings. Containment strategies alongside the development of these modified microorganisms are also required (Bahey-El-Din 2012; Owen et al. 2013).
- Non-colonizing nature of the probiotics due to variable pH in different regions of the gut desires daily dose regimens in large doses (~100 fold higher than injectable dose) to confer specific health benefits to the host. Optimization of parameters associated with microbes (species variability) as well as formulation (protective coatings, novel delivery systems, etc.) must be envisaged in a desirable clinical setting for rendering protection to the microbes inside the gut (Reid et al. 2003; Govender et al. 2014).
- Ideally, development of an edible vaccine on large-scale demands lesser processing. But, lack of the post-translational machinery in the prokaryotes poses a major hurdle and therefore, additional processes further accrue to the cost burden. For the production of the inclusion bodies (IB) as a result of overexpression by the bacterial species of E. coli requires additional processing of unfolding and refolding the proteins into their native state. Number of other post-translational mechanisms may be required according to the desired structural and functional response from the protein antigen and thus establishing a stable post-translational modification protocol during vaccine synthesis is itself a challenge. In fact, research pertaining to post-translational modifications encompasses an array of research collaborations from Medicinal Chemist to Peptide Chemist to Biotechnologist to Formulation Scientists and so on, thereby rendering the scope of discussion far flung (Kopito 2000; Chou 2020).
- Process quality control during the production and formulation of the vaccine product out of these probiotic microorganisms is often cumbersome task. Production of endotoxins is generally observed in the strains of the *E. coli* along with the desired antigen which may be processed and removed to get a desired therapeutic product (Rosales-Mendoza et al. 2016).
- Hygiene and sanitation are the factors that play a major role in the vaccine response. Oral vaccines may produce lesser immune responses in a population

with poor sanitation and increased faecal-oral bacterial exposure. The pathogenic microbiota is responsible for the disrupted immune response.

- Human body has co-evolved in symbiotic relationship with a huge variety of microbiota. Individual variability in the resident microbiota difference in the interaction of the probiotics plays an important role, thereby rendering it difficult to assess proper oral vaccine response.
- Oral vaccines are less responsive in the areas of malnutrition. Findings suggest the role of various nutritional components like vitamins which maintain a proper intestinal function. Improper intestinal health in impoverished and undernourished people may lead to a diminished immune response. Moreover, idiopathic factors like environmental enteropathy which leads to the disrupted intestinal health without any known aetiology are also responsible for the diminished vaccine response (Valdez et al. 2014).
- In case of geriatric patients, the cellular and humoral immune response gets diminished which is responsible for the reduction of the protective effect of the vaccines (Vitetta et al. 2017).
- Regulatory governance of the probiotics as drugs requires stringent protocols for clinical trials for an assessment of utmost factors associated with safety and efficacy. Lack of global regulatory factors for the governing probiotics has created a gap in the developing roadmap for probiotics because the regulations are not same throughout all countries of the world. Global guidelines for probiotics in food have been standardized by various health agencies present across the globe *esp.* World Health Organization (WHO), but the regulatory governance for probiotic drug products demand further enhancement and standardization with coordinated effort at the global level, thereby manifesting a stringent outlook for probiotic based vaccine era to get revealed (Venugopalan2010, Hill et al. 2014, Baldi 2017).

14.7 CRISPR-Cas System (a Prospect in Designing Probiotics as Edible Vaccines)

With an aim of designing strategic vaccines with defined specific immune response, a pursuit to deploy CRISPR (Clustered regularly interspaced short palindromic repeats)-Cas technology in the field of vaccines has already been initiated with many researchers focusing towards the technology. CRISPR and its associated (Cas) proteins are the natural defence systems associated with many of the prokaryotic organisms primarily targeted against bacteriophages and plasmids. The discovery of the functions of the Cas proteins in CRISPR-based gene editing technologies has spurred an arena where the precise modification in the biological machinery of probiotic microorganisms can be rendered possible. Although this genome editing technique is more focused towards eukaryotes, the hidden potential of this technology in the prokaryotes for their industrial applications is yet to be curated out (Barrangou and Doudna 2016; Hidalgo-Cantabrana et al. 2017). With the presence of CRISPR-Cas system in 50% of the bacterial populations, more than half of the

Lactobacilli genomes have been found enriched with the CRISPR-Cas system. In the above context of probiotic research, several genome editing strategies in probiotic bacterial species of Lactobacilli have been applied where the limitations associated in the genome editing were overcome, thereby, further widening the scope of recombinant probiotic designing (Oh and Van Pijkeren 2014; Song et al. 2017; van der Els et al. 2018). The perspectives and goals of designing probiotics with vaccinating potential through oral routes can be made possible by harnessing the potential of CRISPR-Cas technology whereby the following features can be imparted the recombinant probiotics: (a) the threshold/tolerogenic potential to withstand industrial processes with robustness and effectiveness can be enhanced, (b) capability to overcome host environmental parameters like acid and bile tolerance can be imparted for efficient vaccine response, (c) altered metabolic capability to direct the cellular machinery towards broad substrate consumption, (d) surface protein engineered microorganisms as precise and targeted epitope bio-factories, and (e) rendering the non-functional CRISPR-Cas microorganisms functional by heterologous expression (Goh and Barrangou 2019). However, more detailed investigations into the molecular mechanisms need to be rolled out for the precise and specific engineering of the probiotics in the context of their vaccinating potential (Crawley et al. 2018).

14.8 Conclusion

Since their inception to the mankind, probiotics are proven to be of great value, be it in terms of nutrition or medicine. The expanding global population has created a need to combat with pathogenic diseases. The mar of deadly diseases *esp.* in case of endemics or pandemics can be avoided by implementing strategic healthcare planning like mass vaccination. This is possible with the development of economical and easy to administer vaccines. The future research demands the paradigm shift from conventional to oral vaccines. Emergence of concepts on human gut immune system relationship with the microbiome with advancement in molecular biology has put forth a quest among researchers to leverage the potential of probiotics. LAB, *Bifidobacterium, E. coli, Bacillus,* yeasts, etc. are used by mankind from long time, thereby rendering them suitable for oral vaccine development in a befitting manner. Engineering of probiotics as antigen delivery vehicles or immunomodulators is done to specifically target the APCs in the human gut which further elicit a cascade of events for specific immune response. One such advantage of using oral mucosal vaccines is the generation of systemic immune response along with the local mucosal immune response. The advent of research in the field of the CRISPR-Cas systems has further widened the scope of research on probiotics. Harnessing the role of CRISPR-Cas systems in manoeuvring the biological systems of microbes to steer them for antigen production may further fetch a notable research attention. However, in conjunction with an advantageous facet of probiotic's usage, the other facet with the risk of biocontainment, regulatory requirements, multiple dosing, weak immunity, etc., cannot be ruled out. With the successful progress towards the clinical

stage, the scope of developing and using probiotics as oral vaccine seems plausible. Henceforth, the concluding remarks are laid to persuade the researchers to unearth the facets of probiotics as potential candidates for oral vaccine development.

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Probiotics for Atopic Dermatitis

15

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Abstract

This chapter provides information regarding the chronic inflammatory skin disorder atopic dermatitis (AD), highlighting the prevalence of the disease, different diagnostic criteria, diagnosis procedures and clinical features of AD. The pathogenesis of AD is multifactorial resulting from complex interplay among immune dysregulation, epidermal barrier disruption, environment, and genetic predisposition, nutritional, psychological and pharmacological factors. Immune dysregulation and epidermal barrier dysfunction are the major pathophysiological defects along with genetic variation in Filaggrin (FLG), the most recurring finding contributing towards AD development. The major risk factors of AD are positive family history, environmental and lifestyle factors, use of broad-spectrum antibiotics in pregnancy and infants especially *S. aureus* colonisation and superinfections. Sleep disturbance, ADHD, psychiatric disorders, asthma and allergic rhinitis are the co-morbidities associated with chronic skin disease. Though there is no cure for AD, management basically aims at improving symptoms by using therapeutic agents like emollients, calcineurin inhibitors, topical corticosteroids, systemic immunosuppressants and phototherapy depending upon the severity of the symptoms. Gut microbiome impacts AD through immunologic pathway, metabolite pathway and neuroendocrine pathway. Probiotics helps in improving the intestinal barrier by modulating the

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immune status and intestinal microbiome by performing immunomodulatory effect, metabolic effect and standardisation of microbial composition by protection of the mucosal surface against pathogens. This chapter also explores information regarding various studies linking gut and skin microbiome with AD and also summarises the clinical trials using probiotics as interventions in improving the disease condition.

Keywords

Atopic dermatitis · Probiotics · Skin disorder · Dry skin · Gut microbiome

15.1 Introduction

Atopic dermatitis is a chronic inflammatory skin disorder characterised by its clinical hallmarks —xerosis (dry skin), intensive itching and recurring eczematous lesions. This disease is also referred as atopic eczema (Wallach and Taïeb 2014; Weidinger and Novak 2016). Plateauing at 10–20% in the developed countries, this inflammatory disorder still has a growing prevalence in the developing countries (Deckers et al. 2012; Williams et al. 2008). The disease starts affecting the individuals from infancy, manifesting from the first year of life (i.e. early onset) with the earliest clinical signs like dry skin and rough texture but the occurrence of eczematous lesions takes place in the second month or later (Garmhausen et al. 2013; Illi et al. 2004). Atopic dermatitis is equally prevalent in the adults and as the findings suggests a higher prevalent adult onset can start at any age than assumed previously (Weidinger and Novak 2016). Even after long symptom-free periods and outgrowing of disease, the patients tend to suffer from sensitive hyper-reactive skin (Garmhausen et al. 2013). The prevalence of AD in children is age related; being around 60% during the first year after birth and 90% by 5 years of age. The paediatric AD is in rising trend (approximately 30%) in the developing countries and the causes of such trend is still unknown. It may be due to genetic and environmental factors which tend to play a contributing role according to several systematic large-scale studies (Waldman et al. 2018). Various sets of diagnostic criteria like the Hanifin and Rajka criteria (Fig. 15.1) and The UK Working Party criteria (a scientific refinement of the Hanifin and Rajka criteria) are used for clinical and epidemiological studies in children (Brenninkmeijer et al. 2008).

Morphological variations lead to occurrence of clinical features. Dark skinned people tend to suffer from follicular type features mainly characterised by follicular papules that are densely aggregated (Brenninkmeijer et al. 2008; Weidinger and Novak 2016). The lesions are characterised morphologically by erythema, lichenisation, crusting, excoriation and exudation. The AD skin has poor threshold for pruritus and irritation causing an “itch-scratch” cycle leading to secondary infection, poor sleep quality, etc. Environmental irritants, coarse clothing and allergens could lead to exacerbation of pruritus (Waldman et al. 2018). The disease severity depends upon the factors like flare frequency, disease persistence, quality of life and co-morbidities. Several methods are available to delineate the severity of

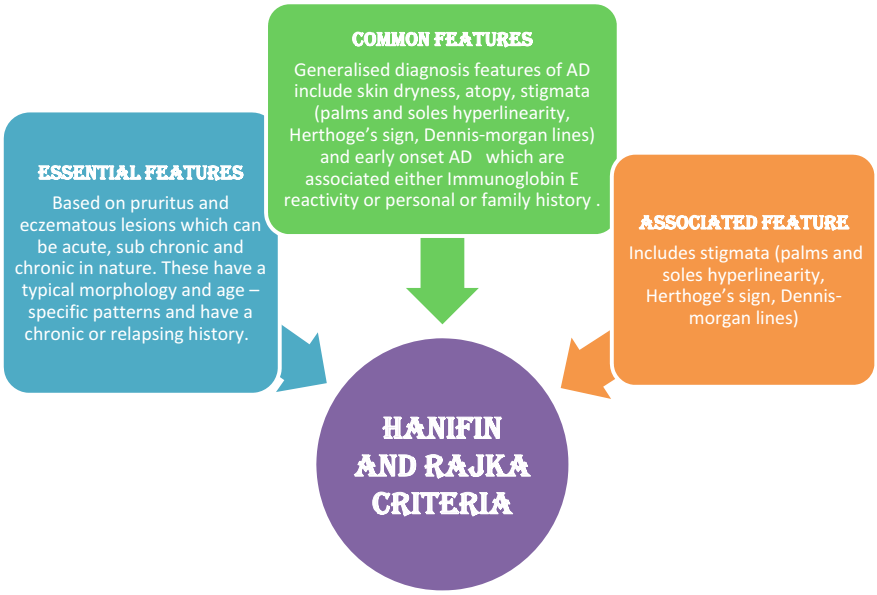

Fig. 15.1 Hanifin and Rajka criteria; one of the prominent diagnostic criteria of atopic dermatitis. This criterion categorises features into: Essential, common and associated features. The figure describes all the varied symptoms that are covered under the different features

AD. Eczema Area Severity Index, Scoring of Atopic Dermatitis Index, Investigator Global Assessment are the preferred scoring systems to measure the signs but are generally not used in the clinical practice (Waldman et al. 2018; Weidinger and Novak 2016). The currently used disease severity scores in clinical trials include: Severity Scoring of Atopic dermatitis (SCORAD), Eczema Area and Severity Index (EASI) (based upon the template of the Psoriasis Area Severity Index (PASI) used for disease severity score for psoriasis) and Harmonising Outcome Measures for Eczema (HOME) (Laird and Lo Sicco 2017). Table 15.1 enlists the common, uncommon and rare occurring clinical disorders that must be first excluded when an individual complains about poor response to therapy, unusual infection or an atypical rash while diagnosing a patient of AD (Weidinger and Novak 2016).

Atopic dermatitis has multifactorial pathogenesis resulting from complex coactions among immune dysregulation, epidermal barrier disruption, environment and genetic predisposition, nutritional, psychological and pharmacological factors (Waldman et al. 2018 7 7). Though, the principal factors and the chief events contributing to the disease still remain the topics of debate. Immune abnormalities and epidermal barrier dysfunction are the two major and converging pathophysiological abnormalities (Elias and Steinhoff 2008). The most steadily replicating findings show involvement of genetic variations in Filaggrin (FLG) in the aetiology of AD. FLG gene copy number variation influences the FLG protein expression that in turn influences AD development. This gene influences factors like skin hydration

Table 15.1 Differential diagnosis of atopic dermatitis

Differential diagnosis	Frequency
Other types of dermatitis	
Seborrhoeic dermatitis	Common
Nummular dermatitis	Common
Irritant contact dermatitis	Common
Allergic contact dermatitis	Common
Lichen simplex chronicus	Uncommon
Asteatotic eczema	Common
Infectious skin disease	
Dermatophyte infection	Common
Impetigo	Common
Scabies	Common[a]
Congenital immunodeficiencies	
Hyper-IgE syndrome	Rare
Wiskott–Aldrich syndrome	Very rare
Omenn syndrome	Very rare
Keratinisation disorders	
Ichthyosis vulgaris	Uncommon
Netherton syndrome	Very rare
Neoplastic disease	
Cutaneous T-cell lymphoma	Uncommon
Nutritional deficiency	
Zinc deficiency	Uncommon

Common = roughly 1 in 10 to 1 in 100; Uncommon = roughly 1 in 100 to 1 in 1000; Rare = roughly 1 in 1000 to 1 in 10,000; Very rare = less than 1 in 10,000

[a]Mainly in developing countries

promotion, barrier function and immune modulation of the superficial epidermis. Immune abnormalities arise majorly due to mechanical injury, microbes and allergy that activate the skin's innate immune system inciting inflammation because of the increased expression of certain cytokines principally IL-25, IL-33 and thymic stromal lipoprotein. Further activation of T-helper (Th-2) cells and increased number of the above-mentioned cytokine release result in suppression of epidermal barrier and antimicrobial peptides (IL-4, IL-5, IL-6) and promotion of eosinophilia, IgE production and cytokine associated inflammation. IL-31 production along with the release of other mediators like neuropeptidases, histamine, tryptase is also promoted by Th-2 release leading to pruritus, one of the most prominent symptoms of AD. In chronic AD, there is also a significant increase of Th-1 and Th-22 cytokines. Structural proteins and lipids that are essential for water retention and barrier protection also show a marked decline impairing the barrier function of the skin significantly (Waldman et al. 2018).

Positive family history of AD forms the major risk factor of the disease (Apfelbacher et al. 2011). This inherited susceptibility is triggered by environmental and lifestyle factors leading to disease manifestation. Some of these environmental risk factors are small family size, western diet enriched with sugar and PUFA, urban

lifestyle, low exposure to ultraviolet radiation and low humidity (Flohr and Mann 2014). Exposure to broad-spectrum antibiotics in pregnant women and infants is also one of the other risk factors of AD (Flohr and Yeo 2011). Patients with AD tend to suffer from various infectious complications due to defect in epidermal barrier and insufficient antimicrobial peptide upregulation. *Staphylococcus aureus* colonisation is observed in up to 90% affected skin patients of AD leading to exacerbation and chronification of disease by producing proteases, releasing enterotoxins and stimulating innate signalling pathways. In number of AD superinfections, Group A *Streptococcus* plays a significant role by increasing the frequency of fever, facial involvement and hospitalisation in children. The combination of factors like aberrant innate and adaptive immune responses as well as epidermal deficiency leads to Herpes simplex virus infection and Eczema herpeticum infection; it is marked by the presence of umbilicated vesicopustules. Children also tend to suffer from ailments like Molluscum contagiosum and eczema coxsackium (caused by coxsackium virus (CVA6))(Waldman et al. 2018).

Co-morbidities associated with AD include sleep disruption, ADHD (attention-deficit/hyperactivity disorder), psychiatric disorders (depression, anxiety and conduct disorders), asthma and allergic rhinitis. Some preliminary studies suggest an increased risk of rheumatoid arthritis, alopecia areata, vitiligo and inflammatory bowel disorder in AD patients (Mohan and Silverberg 2015; Schmitt et al. 2016). On the other hand, decreased risk of type 1 diabetes and cancers (glioma, meningioma and acute lymphoblastic leukaemia) is observed in patients suffering from AD. The disease has some severe effects on patients as well as the society (Deckert et al. 2014; Schmitt et al. 2016). Sleep deprivation, social embarrassment and itch tend to have a major effect on the psychosocial well-being of patients and their relatives (Beattie and Lewis-Jones 2006). Atopic dermatitis has been ranked first amidst common skin diseases with respect to disability-adjusted life years and years lived with a disease in the WHO 2010 Global Burden of Disease survey (Murray et al. 2014; Vos et al. 2012).

As there is no significant cure for AD at present, management of AD aims at improving symptoms and achieving long-term disease control following a multistep approach. The AD management has been outlined in national and international guidelines. Continuous epidermal barrier repair has always been a priority by use of emollients avoiding individual trigger factors. Calcineurin inhibitors and topical corticosteroids are selected classes of drugs for anti-inflammatory therapy. Severely affected cases are managed by systemic immunosuppressants and phototherapy (Weidinger and Novak 2016).

15.2 Relationship between Gut Microbiota and Atopic Dermatitis

Development of AD might witness a crucial role of gut microbiota by regulating the maturation of the immune system due to the crosstalk that occurs between the host and microbiome at the early life (Arpaia et al. 2013; Olszak et al. 2012). “Hygiene

hypothesis" was formulated on the fact that there is an inverse relationship between the early exposure to microbial agents and AD (Derrien and Veiga 2017). Immune system alterations might take place once there is any disruption in the gut microbiome due to the production of metabolites like free phenol and paracresol in vast amounts by the gut flora (Dawson et al. 2011; Zeng et al. 2017). The metabolites tend to affect the distant sites of the organism by entering the circulation and travelling throughout the body. The disrupted epithelial barrier leads to increased intestinal permeability and increasing the levels of metabolite a condition known as "Leaky gut syndrome"(Maguire and Maguire 2017). Figure 15.2 highlights this leaky gut syndrome showcasing disruption of mucosal barrier. According to studies, paracresol and free phenol reduce the expression of keratin 10 in keratinocytes resulting in the disruption of the epidermal barrier integrity.

The mode of delivery greatly influences the microbiota of the skin and gut in the new-borns marking the exposure of microbes during the birth (Dominguez-Bello et al. 2010). A greater association has been found between the caesarean section delivery and an increased risk of immune disorders like asthma, allergy and inflammatory bowel disorders (Sevelsted et al. 2015). Infants acquire microbial species typically from the mother's skin surface in which *Staphylococcus, Corynebacterium* and *Propionibacterium* sp. dominantly found on the mother's skin. On the other hand, vaginally delivered infants harbour bacterial communities of general *Lactobacillus* and *Prevotella* resembling their mother's vaginal microbiota, *lactobacilli* of such origin acts as a protective barrier of the immature immune system of the infants against pathogens that have a major relevance in skin disorders like *Staphylococcus aureus* (Dominguez-Bello et al. 2010). In early life, stress, diet and pollution are amongst the various environmental factors affecting the composition and profiling of the microbes; such diverse factors drive the contribution of the gut microbiota in the development of AD (Gensollen and Blumberg 2017). Damage of intestinal barrier is noted in individuals consuming dietary gluten; even they do not suffer from celiac disease leading to a leaky gut (Uhde et al. 2016). Severe cutaneous manifestations mimicking AD has been further associated with both celiac and non-celiac gluten sensitivity (Bhatia et al. 2014; Bonciolini et al. 2015). Severity of AD can also be correlated to the low Vitamin D levels, as the systemic Vitamin D metabolism is regulated by the gut microbiota and acts as a significant signalling mechanism between the host and microbiota (Bora et al. 2018; Ly et al. 2011). The proportion of *Bifidobacteria, Bacteroides* and *Bacteroidetes* decreases significantly in the patients of AD as compared to control; on the other hand, *Clostridium difficile, Clostridia, Escherichia coli* and *Staphylococcus aureus* tend to show a higher number in the gut microbiome. Table 15.2 shows such alterations in microbial diversity in non- affected AD skin v/s lesional AD skin (Abrahamsson et al. 2012; Adams et al. 2006; Kirjavainen et al. 2002; Lee et al. 2016; Nylund et al. 2015). Short-chain fatty acids (SCFA) such as butyrate, acetate, lactate and propionate tend to exert anti-inflammatory effect as well as maintain the epithelial barrier integrity (Smith et al. 2013). These SCFA are produced during the fibre fermentation by the gut microbiota (Maslowski et al. 2009). Species associated with Firmicutes phylum such as *Roseburia intestinalis, Eubacterium hallii* and *Faecalibacterium prausnitzii*

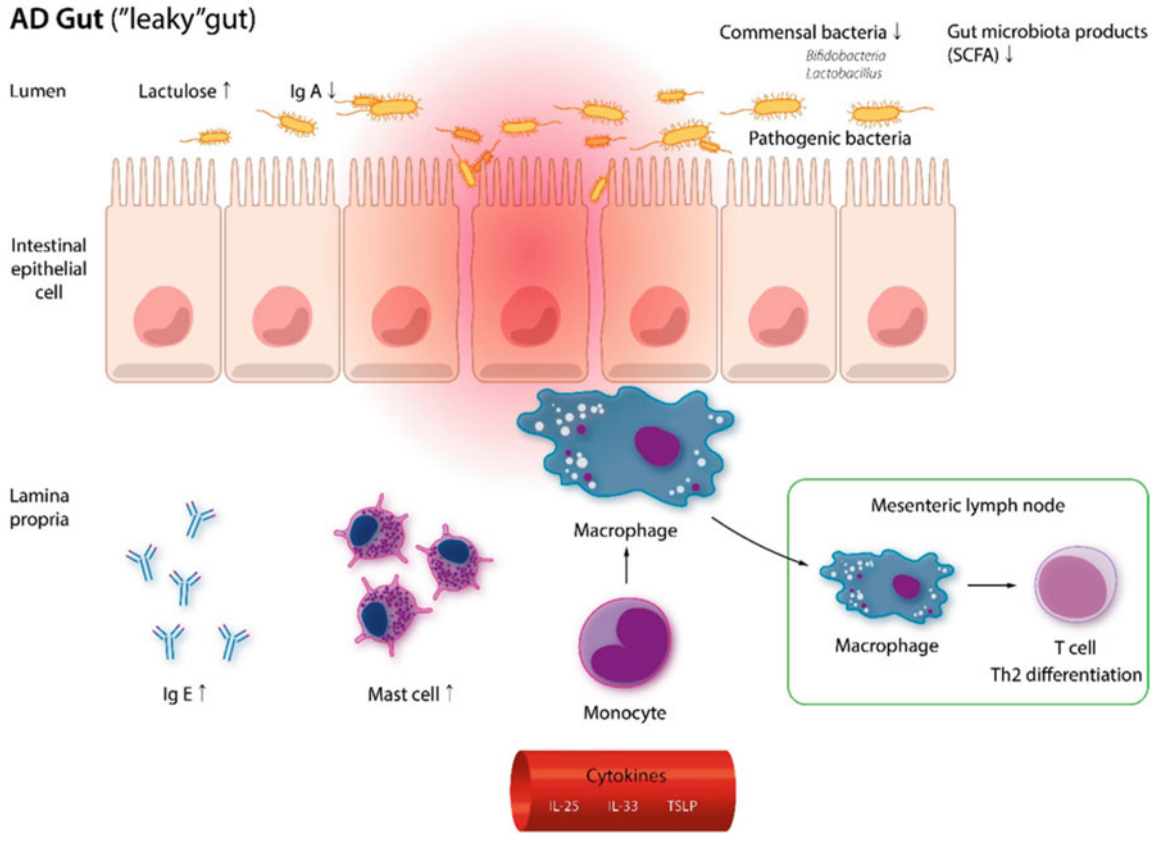

Fig. 15.2 Disruption of mucosal barrier in AD. Patients with AD have dysbiosis and less short-chain fatty acids (SCFAs) in the gut. In response to proinflammatory cytokines, monocyte migrate and differentiate into macrophages, greater access to luminal antigens also causes T-cells to transform into Th-2 cells in the draining lymph nodes. Immunoglobulin E (Ig E) and mast cells are also more abundant in lamina propria. *IgE* immunoglobulin E, *IL* interleukin, *Th2* T-helper cells, *TSLP* thymic stromal lymphopoietin, *IgA* immunoglobulin A (Kim and Kim 2019)

Table 15.2 Alterations in microbial diversity in non-affected AD skin v/s lesional AD skin (Kim and Kim 2019)

Non- Affected AD skin	Lesional AD skin
Firmicutes (phylum) *Streptococcus* (genus) *Staphylococcus* (genus) *Granulicatella* (genus)	*Streptococcus* and *Granulicatella* had decreased relative abundance. *Staphylococcus* had increased relative and absolute abundance.
Bacteroides (phylum) *Prevotella* (genus)	Relative abundance decreased
Proteobacteria (phylum) *Acinetobacter* (genus)	Relative abundance decreased
Actinomyces (genus) Actinobacteria (phylum) *Corynebacterium* (genus) *Cutibacterium* (genus) *Rothia* (genus)	Relative abundance decreased

are involved in the production of butyrate (Louis and Flint 2017). Propionate is produced by the species belonging to Bacteroidetes phylum like *Bacteroides uniformis, Prevotella copri* and Verrucomicrobia phylum like *Akkermansia muciniphila* in the patient of AD (Louis and Flint 2017; Mikó et al. 2019), indicating the gut microbiome playing role in the SCFA pathway was noted to have increased significantly. Association of AD with intestinal *Clostridia* and *Escherichia coli* is established due to eosinophilic inflammation leading to the development of AD (Lee et al. 2016). Development of host immune system is also disrupted due to the alterations of functional genes in the presence of specific gut microbiome like *Akkermansia muciniphila* and *Ruminococcus gnavus* (Song et al. 2016).

Secondary bile acids like the litocholic acid and deoxycholic acid produced by Firmicutes and Bacteroidetes phyla also reported to have impacts on the physiology of the skin (Mikó et al. 2018; Ridlon et al. 2006). Adaptive immune response is greatly affected due to faulty activation of Th-1 cells reportedly caused by litocholic acid (Pols et al. 2017). Reports show that *Clostridium difficile* infection that affects the skin in the secondary bile acid dependent manner could confer resistance in the presence of *Clostridium scindens* belonging to Firmicutes phyla (Buffie et al. 2015). The combined effects of gut microbiome dynamics and varied environmental factors should be studied further for more accurate evaluation of the impact of gut microbiome in AD development.

15.3 Mechanism Involved

Gut microbiome impacts AD through three major pathways, namely immunologic, metabolite and neuroendocrine pathways (Fig. 15.3) (Kim and Kim 2019).

15.3.1 Immunologic Pathway

Barrier dysfunction and immune response are two major biological pathways involved in the clinical manifestation of AD. Secretion of T-helper (Th) 2 cytokines like IL-4, IL-5 and IL-13 due to TH1/TH2 imbalance leads to increased production of immunoglobulin E (IgE) and increased binding of *S. aureus* to AD infected skin. Being the most common pathogen affecting AD akin, *S. aureus* carries a combination of superantigens and adhesion genes that affects AD development in infancy negatively by stimulating and promoting the immune system of infants. Although *S. aureus* leads to increase in the severity of established AD, commensal mucosal colonisation by *S. aureus* before the "atopic march" could provide a possibility of a broad immune stimulation by this bacterium generating a protective effect (Huang et al. 2017).

15.3.2 Metabolite Pathway

The relationship between dietary supplements, microbiome and immune system of skin can be established by the SCFAs released by the gut microbiome, majorly by *Akkermansia muciniphila* playing a significant role in various inflammatory diseases like AD (Reichardt et al. 2014; Song et al. 2016; Thorburn et al. 2014). Various studies supported that the anti-allergic and anti-inflammatory effects due to the oral administration of the metabolites can modulate various skin diseases (Miyamoto et al. 2015). Alleviation of AD and gut microbiome modulation in a mouse model was observed after the administration of linoleic acid and 10-hydroxy-cis-12-octadecenoic acid. While in another study, reduced scratching behaviour in AD mice due to administration of probiotic *Bifidobacterium animalis* subsp. *lactis* (LKM512) was observed, which was attributed to increased levels of the metabolite kynurenic acid (Lee et al. 2018b; Matsumoto et al. 2014). These diverse studies support that there is an existence of gut-skin axis communications mediated by metabolites.

15.3.3 Neuroendocrine Pathway

Neuroendocrine molecules produced by the gut microbiome show effects on the gut-skin axis. The degree of AD symptoms depends on the release of diverse neuromodulators and neurotransmitters, now being associated to differences in composition and proportion of gut microbiome. These neuromodulators and

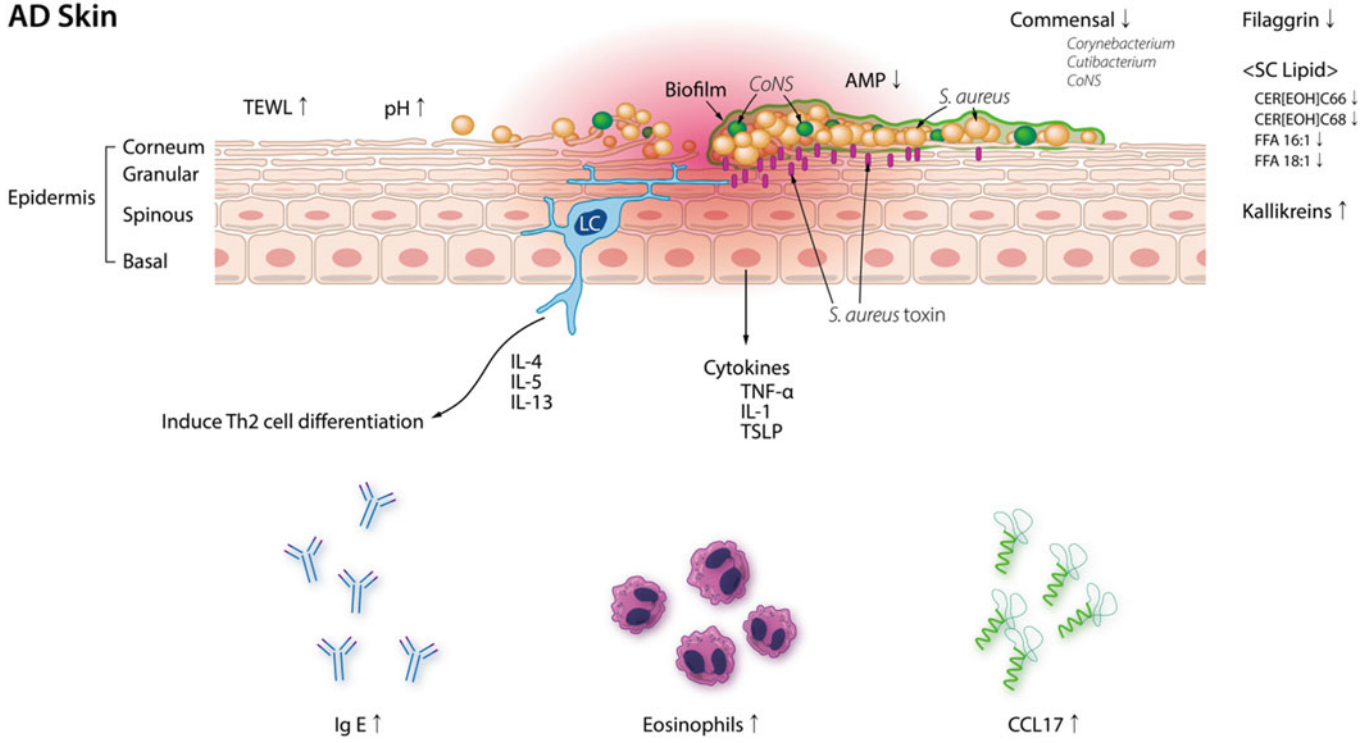

Fig. 15.3 Disruption of epidermal barrier in the AD skin. Trans-epithelial water loss (TEWL), pH, serum IgE, serum thymus and activated cytokine (TARC/CCL17), and eosinophils are significantly elevated in AD patients. Filaggrin and stratum corneum (SC) lipid composition, and serine protease (Kallikreins) are also altered in AD, allowing *S. aureus* colonisation. With decrease in coagulase-negative *Staphylococci* (CoNS) and its antimicrobial peptides (AMP), *S. aureus* proliferates and also forms biofilms. *AD* atopic dermatitis, *CER* ceramide, *FFA* free fatty acid, *IL* interleukin, *S. aureus* staphylococcus aureus, *TNF* tumour necrosis factor, *TSLP* thymic stromal lymphopoietin (Kim and Kim 2019)

neurotransmitters affect the immune system dysregulation and skin barrier dysfunction, which are the two key pathophysiologies in AD development (Lee et al. 2018b). Direct and indirect pathways are undertaken by the gut microbiome in order to modulate the gut-skin axis (Yokoyama et al. 2015). Tryptophan released by the gut microbiome directly affects the gut-skin axis by producing an itching sensation in the skin (Jin et al. 2014). Skin pigmentation can occur by serotonin produced by *Escherichia* and *Enterococcus* species (Cryan and Dinan 2012; Lee et al. 2011). Indirect pathways involve the release of cortisol under stress conditions that could alter the gut microbiome composition leading to changes like alteration in barrier function and gut epithelium permeability (Cryan and Dinan 2012). Cytokine levels in the bloodstream can also be modulated indirectly by gut microbiome affecting anxiety and stress, further affecting the levels of cortisol (Yokoyama et al. 2015).

S. aureus colonisation takes place due to alterations in filaggrin and stratum corneum lipid composition as well as serine protease, i.e. kallikreins. Proliferation of *S. aureus* and formation of biofilms occur due to decrease in Coagulates-negative *Staphylococci* (CoNS) and its associated antimicrobial peptides (AMP). AD patients show an elevation in pH, eosinophils, serum IgE, activated cytokine (TARC/CCL17), serum thymus and trans-epithelial water loss (TEWL) (Kim and Kim 2019). Probiotics help in improving the intestinal barrier by modulating the immune status and intestinal microbiome that further helps in reducing the allergic phenomenon as well as AD severity (Alvarez-Olmos and Oberhelman 2001). There are increasing number of studies being conducted in recent years to evaluate the effect of probiotics administration in patients at different stages of their life, i.e. during pregnancy and lactation, infancy, childhood and adulthood (Rusu et al. 2019). Studies suggest that probiotics contributed significantly in combating the severe symptoms of atopic dermatitis and providing efficient and beneficial treatment to the patients suffering from AD.

Probiotics follows different mechanism of actions in order to provide relief to the inflammatory skin disorder (Kim and Kim 2019) (Fig. 15.4).

15.3.4 Immunomodulatory Effect

Probiotics act by stabilising the Th1/Th2 ratio reducing the severity of AD. They also inhibit the Th-2 mediated response, further leading to decreased or no release of the cytokines (IL-4, IL-5, IL-6 and IL-13) (Enomoto et al. 2014; Feleszko et al. 2007; Jang et al. 2012; Nwanodi 2018). There is a significant reduction in the proinflammatory cytokines, tumour necrosis factor-a (TNF-a), IL-4, IL-6, INF gamma and high sensitivity C reactive protein (hsCRP) (Zheng et al. 2018) and a remarkable increment in the expression of Treg-related cytokines and IL-10 at the mesenteric lymph nodes leading to reduced inflammation contributed by the probiotics (Rusu et al. 2019). The efficacy of probiotics was suggested in a new mechanism where its ability to inhibit the mature dendritic cell differentiation and transforming of naive T cells into Th-2 is demonstrated (Kim et al. 2013). Probiotics

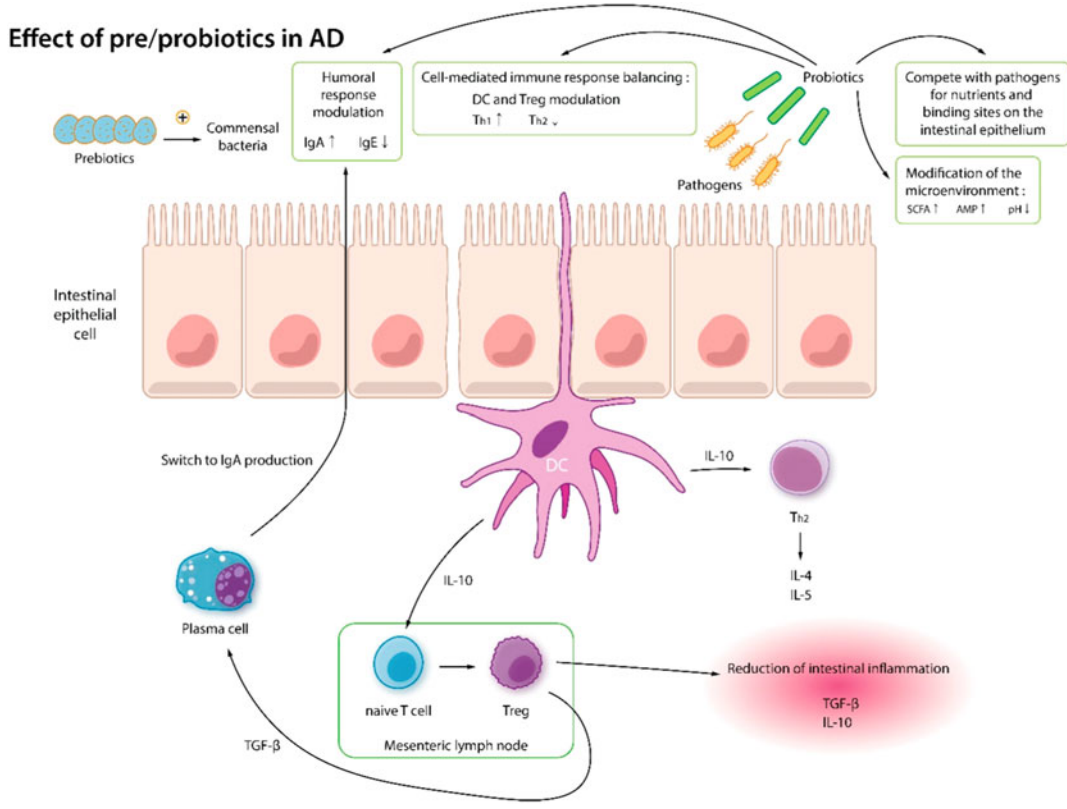

Fig. 15.4 Immune mechanisms of Pre- and probiotics. Probiotics and commensal bacteria when fed along with prebiotics, probiotics seem to modulate the microenvironment, competing with pathogens, balancing the cell mediated immune response (decreases response of Th2, increases Treg cells) and humoral response (increases IgA and decreases IgE). *SCFA* short-chain fatty acid, *AMP* antimicrobial peptide, *DC* dendritic cells, *IL* interleukin, *TGF* tumour growth factor, *IgE* immunoglobulin E, *IgA* Immunoglobulin A, *Th1* T-helper 1 cells, *Th2* T-helper 2 cells (Kim and Kim 2019)

promote phagocytosis; stimulate the release of IL-10 and transforming growth factor-b (TGF-b) as well as increases level of serum IgA (Tatu et al. 2016). Probiotics also modulate the intestine-skin axis and brain function reducing the susceptibility to inflammatory and allergic factors and stress to the intestine-skin axis, respectively (Messaoudi et al. 2011; Rusu et al. 2019).

15.3.5 Protection of the Mucosal Surface against Pathogens by Standardisation of the Microbial Composition

The prevalence of allergic diseases is influenced by the abundance of varied *Bifidobacterium* species in the faeces of the newborn. A notable higher level of *Bifidobacterium longum* was observed in healthy children when a study was conducted to detect the levels of varied *Bifidobacterium* species in the faeces of allergic children as compared to healthy ones proposing the role of this strain in avoiding the occurrence of allergic dermatitis and bronchial asthma (Akay et al. 2014). *Lactobacillus* is also amongst the popular probiotic agent providing benefits like an acceleration of the skin barrier recovery as well as inhibiting inflammation of skin related to substance P (Gueniche et al. 2010). Human origin *Lactobacillus* along with *Enterococcus* also indicated that probiotics could lead to the strengthening of mucosal barrier as it increases the production of SCFA (Nagpal et al. 2018; Salem et al. 2018). In fact, modulation in the microbial composition can also occur by using probiotics as a therapeutic tool.

15.3.6 Metabolic Effect

Proinflammatory cytokines (IL-6, TNF-a, CRP), chronic systemic inflammation, inflated oxidative stress and changing expressions of inflammatory genes establish a relationship between AD and metabolic disorders like obesity, dyslipidaemia, etc. (Rusu et al. 2019). There is an association between probiotic consumption and reduced blood glucose, insulin resistance and insulinaemia (Ruan et al. 2015). Various meta-analysis studies were also conducted to collect more evidences relating the probiotics and metabolic effect. One study was conducted in patients suffering from non-alcoholic fatty liver disease where the decrease in alanine aminotransferase and insulin resistance showed favourable effects (Ma et al. 2013). Other suggested the association of decreased total cholesterol, triglycerides, low density lipoprotein cholesterol and increased high density lipoprotein cholesterol with administration of probiotics (He et al. 2017). Overweight and obese patients had a higher risk of AD development, highlighted by the systematic review conducted by Zhang and Silverberg (Zhang and Silverberg 2015).

15.4 Clinical and In-Vivo Status

There have been various studies that show the involvement of both gut microbiome and skin microbiome in AD. Table 15.3 summarises previous studies that report the involvement of gut microbiome in AD. Table 15.4 summarises the previous studies of involvement of skin microbiome in AD.

COCHRANE review assessed the effects of probiotics on the patients with eczema and examines the advantageous effects of probiotics on the AD patients (Hulshof et al. 2017). According to the review, no evidence was found suggesting that probiotics showed any major difference in the quality of life of eczema patients. Even little or no difference was made by probiotics in the participant or patient related symptoms that were associated with eczema. The review also indicates reduced investigator rated eczema severity scores but clinically the results were not sufficient. Above findings concluded that currently usage of probiotics for the treatment of eczema is not evidence based. The variance in response is observed as species and strains employed in the treatment have significant variations, thus Meta-analysis does not support the use of probiotics for treatment of AD (Hulshof et al. 2017).

Randomised control trials also took place as the results of past clinical interventions showed inconclusive results of probiotics in AD treatment (Hulshof et al. 2017). Four randomised clinical trials conducted by Han et al. (2012), Woo et al. (2010), Miniello et al. (2010) and Torii et al. (2011) provided results of study using only one probiotic as dietary intervention (Table 15.5) (Han et al. 2012; Miniello et al. 2010; Torii et al. 2011; Woo et al. 2010). AD severity was improved in three out of four trials using one probiotic strain (Hulshof et al. 2017). Additional effect was observed in the (Han et al. 2012) clinical trial, the cytokine level of IL-4 and IFN-y significantly lowered or did not fluctuate as was changing before the intervention. On the other hand, two randomised clinical trials conducted by Wang and Wang (2015) and Gore et al. (2012) used more than one probiotic strain in the study (Table 15.6) (Gore et al. 2012; Wang and Wang 2015). Gore et al. trial revealed a reduction in SCORAD score in both the groups, whereas (Wang and Wang 2015) reported that selected AD children having at least one elevated specific IgE level or at least one positive skin prick test showed improvement in AD with probiotic administration. Both these studies showed IgE level changes but there was no significance between treatment and control (Hulshof et al. 2017).

The above-mentioned clinical trials have several confounding factors that should be considered while interpreting the results of the study. For example, SCORAD score is used and validated for clinical outcome comparisons in order to assess the AD severity but such scoring is difficult when noted in infants due to inter-observer variability and the constant relapsing–remitting nature of AD in young patients complicates the process of assessing AD severity and nutritional intervention effects at a particular time (Hulshof et al. 2017). AD severity recommendations for future clinical trials were made based on evaluation using core symptom instruments such as SIS (skin intensity score), POEM (patient–oriented eczema measure), SA-EASI (self-administered eczema area and severity index score), SCORAD (severity

Table 15.3 Summary of the previous studies reporting the involvement of gut microbiome in AD (Lee et al. 2018b)

Author and Year	Research title	Subjects	Methods	Observations
Penders et al. (2007)	Gut microbiota composition and development of atopic manifestations in infancy: KO-ALA birth cohort study	Total no. of subjects taken (n) = 957	qPCR	Presence of *E. coli*: Risk of developing eczema becomes higher Presence of *C. difficile*: Recurrent wheezing, allergic sensitisation and risk of developing eczema becomes higher
van Nimwegen et al. (2011)	Mode and place of delivery, gastrointestinal microbiota and their influence on asthma and atopy	Age: 1 month(n = 1176) 1 year (n = 921) 2 year (n = 822) 6–7 years(n = 384)	Quantitative PCR	*C. difficile* colonisation governed the effects of mode and place of delivery on atopic outcomes as was shown by the mediation analysis.
Abrahamsson et al. (2012)	Low diversity of the gut microbiota in infants with atopic eczema	AD infants (n = 20) Healthy until age 2 (n = 20), at 1 week, 1 month, 12 months of age.	16S pyrosequencing	AD infants at 1 month of age showed decreased diversity of total microbiota. Ad infants at 1 month showed decreased diversity of bacteroidetes, bacteroides and at 12 month showed decrease in Proteobacteria diversity. Healthy infants showed increased Proteobacteria until the age 2
Penders et al. (2013)	Establishment of the intestinal microbiota and its role for AD in early childhood	Probiotic supplementation to the individuals ages between 5 weeks to 7 months. 5 weeks (n = 571) 13 weeks(n = 332) 31 weeks(n = 499)	qPCR	Microbiota composition was established according to order and mode of birth as well as breast feeding. Older sibling: Increased *Lactobacilli* and Bacteroides and decreased clostridia at 5 weeks of age. Individuals with AD showed increased clostridia at 5 and 13 weeks of age

(continued)

Table 15.3 (continued)

Author and Year	Research title	Subjects	Methods	Observations
Nylund et al. (2015)	Severity of atopic disease inversely correlates with intestinal microbiota diversity and butyrate-producing bacteria	Healthy infants ($n = 11$) Infants with AD ($n = 28$)	16S rRNA gene Microarray	Microbiota diversity and plethora of butyrate-producing bacteria are inversely related to the severity of eczema.
Lee et al. (2016)	Clostridia in the gut and onset of AD via eosinophilic inflammation	6 months of age AD infants ($n = 12$) Healthy infants($n = 12$)	16S rRNA pyrosequencing	The two groups showed no significant differences in the diversity of gut microbiota Inverse correlation was established between clostridia and blood eosinophil (%) Weak correlation was established between bacilli, *E. coli* and blood eosinophil (%) Later development of AD was due to increased clostridia.
Song et al. (2016)	*Faecalibacterium prausnitzii* subspecies-level dysbiosis in the human gut microbiome underlying AD	6 months of age AD infants ($n = 90$) Healthy infants($n = 42$)	16S rRNA gene and metagenome sequence analyses	Increased genes lead to the association of the enrichment of the subspecies of major gut species *F. prausnitzii* with AD microbiome present in the AD infants
Lee et al. (2018a)	Perturbations of the gut microbiome genes in infants one with AD	AD infants ($n = 63$) Healthy infants($n = 66$)	16S rRNA gene and whole metagenome sequencing	Decreased colonisation of mucin degrading bacteria in AD infants lead to induction genes for PI3-Akt signalling, oestrogen signalling, NOD-like receptor signalling, oxidative phosphorylation and antigen processing and presentation.

Table 15.4 Summary of the previous studies involving skin microbiome in AD (Lee et al. 2018b)

Author and year	Research title	Subjects	Methods	Observations
Kong et al. (2012)	Temporal shifts in the skin microbiome associated with disease flares and treatment in children with AD	AD patients ($n = 12$) Healthy controls($n = 10$)	16S rRNA genes	Increased proportion of *S. aureus* and *S. epidermidis* in AD lesions
Oh et al. (2013)	The altered landscape of the human skin microbiome in patients with primary immunodeficiencies	AD patients ($n = 13$) Healthy controls ($n = 46$)	16S rRNA genes	*S. aureus* population increases in AD Human skin microbiome was positively correlated with the severity of disease. Relatively lower diversity was observed as compared to healthy controls.
Laborel-Préneron et al. (2015)	Effects of *S. aureus* and *S. epidermidis* secretomes isolated from the skin microbiota of atopic children on CD4+ T cell activation	AD patients ($n = 21$; mean ages = 24.1 months) Healthy controls ($n = 17$; mean ages = 24.9 months)	RT-PCR analysis of skin scratches	Increase in *S. aureus* population indicates towards AD Associated with the interference in the activation of Dentritic cells and CD4 + T cell differentiation from monocytes leading to greatest total IgE levels and CD4 + T cell response. Treg cells experience direct inhibitory response.
Shi et al. (2016)	The skin microbiome is different in paediatric versus adult AD	AD patients ($n = 128$; Young children = 59, teenagers =13, adults = 56) Non-atopic age matched healthy controls ($n = 68$; Young children = 13, teenagers = 10, adults = 45)	16S rRNA gene sequencing	Increase in *Streptococcus, Granulicatella, haemophilus, Rothia, Gemella* was observed in young children. Increase in *Propionibacterium, Finegoldia, Corynebacterium, Staphylococcus, Lactobacillus,*

(continued)

Table 15.4 (continued)

Author and year	Research title	Subjects	Methods	Observations
				Anaerococcus was observed in adults
Drago et al. (2016)	Skin microbiota of first cousins affected by psoriasis and AD	Three male's first cousins with age ranging between 47 and 53 years.	16S rRNA gene amplification	The subjects suffering from psoriasis showed decline in Firmicutes whereas a significant increase in *Proteobacteria, Streptococcaceae, Campylobacteraceae, Rhodobacteraceae and Moraxellaceae* was observed Psoriatic individuals, healthy control and AD non-lesional skin showed very similar microbial composition.
Kennedy et al. (2017)	Skin microbiome before development of AD: Early colonisation with commensal *staphylococci* at 2 months is associated with lower risk of AD at age 1	50 infants at 3 points in the first 6 months of life at 4 sites AD patients ($n = 10$) Healthy controls ($n = 10$)	16S rRNA gene sequencing	Reduced development of AD and modulation of skin immunity at 12 months observed in two-month antecubital fossa samples of commensal *staphylococci (S. epidermidis, S. cohnii)* Markedly less abundance of commensal staphylococci was observed in infants suffering from AD at 12 months.
Nakatsuji et al. (2017)	Antimicrobials from human skin commensal bacteria protect against *S. aureus* and are deficient in AD.	AD patients ($n = 49$; mean age = 33.4 + _ 14.1 years) Non-AD individuals ($n = 30$; mean age = 33.9 + _18.2 years)	16S rRNA community sequencing	Increase in *S. epidermidis* and *S. hominis* was observed leading to increase in strain specific AMP and selectively eliminating *S. aureus*

Kim et al. (2017)	A metagenomic analysis provides culture independent pathogen detection for AD.	AD patients ($n = 27$) Healthy controls ($n = 6$)	High throughput pyrosequencing on a Roche 454 GS-FLX platform	Increase in *Staphylococcus, Pseudomonas* and *streptococcus* was observed in AD skin lesions Increase in *Alcaligenaceae* (f), *Lactococcus* and *Sediminibacterium* was observed in cubital fossa of healthy controls.

Table 15.5 Randomised control trials using one probiotic as dietary intervention

Reference	Subjects (Age, *N*, Treatment v/s control)	Inclusion criteria	Dietary intervention	Treatment period and dose of probiotics	Primary parameter	Clinical outcome, AD severity and IgE	Immunological outcomes
Han et al. (2012)	Children (1-13 years) $N = 83$ 44 v/s 39	SCORAD ranging between 20 and 50	*Lactobacillus plantarum* (LP) CJLP133 Control diet; preparation of placebo No fermented food products containing live microorganisms were allowed	Twice daily; for 12 weeks LPCJLP133 $0.5*10^{10}$ CPU	Clinical and immunological paramaters should show significant improvement in AD children	Significant decrease in SCORAD score as compared to control ($p = 0.004$) after dietary intervention	After dietary intervention, significantly lower total eosinophilic count and logarithmic IFN-y and IL-4 were observed as compared to the control ($p = 0.023$), ($p < 0.001$), ($p = 0.049$)
Woo et al. (2010)	Children (2–10 years) $N = 75$ 41 v/s 34	Prior to study AEDS for 6 months Total SCORAD>25	Microcrystalline cellulose with *Lactobacillis sakei* (LS) KCTC 10755BP Control diet; only microcrystalline cellulose	Twice daily; for 12 weeks LSKCTC 10755BP 5×10^9 CFU	AD clinical outcome evaluation	Significant greater mean change in Total SCORAD of the probiotic group compared to the control group ($p = 0.008$)	Probiotic group showed a significant decrease in serum CCL17 and CCL27 levels as compared to control (both $p < 0.001$)
Miniello et al. (2010)	Children (4–10 years), $N = 51$, 26 v/s 25	AD symptoms Prior to study, no antibiotics and no use of local	Chewable tablets with *Lactobacillus reuteri* (LR) ATCC53730 Control diet;	One daily; for 8 weeks LR ATCC53730 1×10^8 CFU	Changes in exhaled breath condensate (EBC) cytokine expression.	No significant changes in the SCORAD mean values of probiotic and control groups	Significant decrease in IL-4 and increase in EBC IFN-y was observed in 16 IgE positive children of

		corticosteroids for 8 weeks	chewable placebo tablet			after the dietary interventions	probiotic group as compared to 14 IgE positive children of the control group.
Torii et al. (2011)	Children (4–15 years), $N = 20$	AD, No cow's milk, Spec Ig E	Fermented milk with *Lactobacillus acidophilus (LS)* L-92 No control diet	One daily; for 8 weeks *Lactobacillus acidophilus* LSL-92 3×10^{10} CFU + 150 ml milk	Estimation of symptom medication score (SMS) that is evaluated as the sum ADASI and medication score of less topical steroid use.	Changes were observed in ADASI, SMS and itch (all three: $p < 0.01$)	Blood biochemical parameters including total plasma IgE concentration remains unchanged

Table 15.6 Randomised clinical trial using more than one probiotic strain in the study

Reference	Subjects (Age, *N*, Treatment v/s control)	Inclusion criteria	Dietary intervention	Treatment period and dose of probiotics	Primary parameter	Clinical outcome, AD severity and IgE	Immunological outcomes
Gore et al. (2012)	3–6 months >34 weeks gestation $N = 137$ 90 v/s 47	>200 ml standard formula daily SCORAD>10	Extensively hydrolysed formula with a sachet *Bifidobacterium lactis* (BL) CNCM I-3446 or with a sachet *Lactobacillus paracasei* (LP) CNCM I-2216. Control diet; formula of extensively hydrolysed with maltodextrin sachet	For 12 weeks; (LP) CNCM I-2216: 10^{10} CFU; $N = 45$ (BL) CNCM I-3446: 10^{10} CFU; $N = 45$ Control $N = 47$	Change in SCORAD	In all groups, over time SCORAD reductions decreases significantly after dietary intervention	Post intervention probiotic treatment showed no significant effect on the prevalence of allergen sensitisation.
Wang and Wang (2015)	Children (1–18 years), $N = 220$ 165 v/s 55		Capsule with *Lactobacillus fermentum* (LF)GM090 or capsule with *Lactobacillus paracasei* (LP)GMNL-133 or capsule with both probiotics Control diet; placebo capsule	For 3 months; (LP)GMNL-133: 2×10^9 CFU; $N = 55$ (LF)GM090: 2×10^9 CFU; $N = 53$ (LP) GMNL-133 + (LF) GM090: 4×10^9 CFU; $N = 51$ Control $N = 53$	Change in AD severity	Lower SCORAD compared to control ($p < 0.001$) after dietary intervention. After discontinuing the probiotics, difference remained at four months.	LP and LP + LF groups showed reduced total IgE levels, as compared to control there was no significant difference. IL-4 changed significantly as compared to control ($p = 0.04$)

scoring of atopic dermatitis index) and adapted SA-EASI. Mucosal immune system programming and prevention of AD development via microbial modulation is basically preferred in the first year of life. Though dietary intervention studies during pregnancy, lactation or in the early life of infants revealed decreased risk of AD development but tend to have no effect on the development of other allergies unlike some of the meta-analysis studies justifying the clinical evidence of dietary intervention in AD. Due to inconsistent results obtained from various studies substantial evidence is still low. In order to maintain the consistency in the various studies, a defined criterion should be set up for easy comparability of the clinical trial outcomes. Such consistency in studies subsequently motivates to provide reliable advice for implementation of dietary interventions in AD management (Gerbens et al. 2018).

15.5 Conclusion

This chapter provides elaborate information on the chronic inflammatory skin disorder, atopic dermatitis (AD), highlighting the prevalence of the disease, different diagnostic criteria and differential diagnosis tools of AD. The pathogenesis of AD is multifactorial resulting from complex interplay among immune dysregulation, epidermal barrier disruption, environment, genetic predisposition, nutritional, psychological and pharmacological factors. Immune abnormalities and epidermal barrier dysfunction are the major pathophysiological abnormalities along with genetic variation in FLG play significant role in AD development. The major risk factors of AD are also enlisted with various co-morbidities associated with the disease. AD management basically aims at combating symptoms by using therapeutic agents like emollients, calcineurin inhibitors, topical corticosteroids, systemic immunosuppressants and phototherapy depending upon the severity of the symptoms, as there is no significant cure of AD.

There is an established relationship between the gut microbiome and atopic dermatitis. Gut microbiome impacts AD through immunologic pathway, metabolite pathway and neuroendocrine pathway. Stress, diet and pollution are amongst the various environmental factors that affect the composition and proliferation of the microbes leading to development of AD. Low vitamin D, short-chain fatty acids (SCFA) and secondary bile acids like the litocholic acid and deoxycholic acid play different roles in gut dysbiosis and impacting epithelial barrier integrity and physiology of the skin, respectively. Probiotics improves the intestinal barrier by modulating the immune status and intestinal microbiome by performing immunomodulatory, metabolic effect and protection of the mucosal surface against pathogens by standardisation of the microbial composition. The chapter in the end summarises various studies linking gut and skin microbiome with atopic dermatitis. It also summarises different clinical trials reporting the role of probiotics as dietary intervention in improvement of disease condition.

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