Indu Pal Kaur *Editor-in-Chief*
Parneet Kaur Deol *Editor*

Probiotic Research in Therapeutics

Volume 1: Applications in Cancers and Immunological Diseases

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Editor-in-Chief
Indu Pal Kaur
Panjab University
Chandigarh, Punjab, India

Editor
Parneet Kaur Deol
G.H.G. Khalsa College of Pharmacy
Gurusar Sadhar
Ludhiana, Punjab, India

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To my father who taught me to think and write rationally.
To my mother from whom I get my confidence and the feature to express clearly.
To my husband who encouraged me to grow professionally and enjoy finer things in life.

—Prof. Indu Pal Kaur

To the four pillars of my life—my husband, my daughter, my parents, and my GURU.

—Dr. Parneet Kaur Deol

Foreword

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 lifestyle 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 toward 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 the 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 but surfing through scientific claims on the usefulness of probiotic therapy can catch the fancy of even a general reader.

I have known Prof. Indu Pal Kaur, Editor-in-chief 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 lifestyle diseases, such as depression, chronic fatigue syndrome, vaginal candidiasis, wound healing, and skin health.

The present e-book series, comprising five volumes, brings the latest information and key insights on the 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 ensures 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!

J. V. Yakhmi
Asia Pacific Academy of Materials
Mumbai, Maharashtra, India

Homi Bhabha National Institute
Mumbai, Maharashtra, India

Physics Group, Bhabha Atomic Research Centre
Mumbai, Maharashtra, India

Technical Physics and Prototype Engineering Division,
Bhabha Atomic Research Centre
Mumbai, Maharashtra, India

Spectroscopy, Synchrotron and Functional Materials,
Bhabha Atomic Research Centre
Mumbai, Maharashtra, India

Preface

Human beings are metaorganisms comprising the macroscopic host and coexisting plethora of symbiotic commensal organisms. A vast majority of these microorganisms coinhabit within the confines of the gastrointestinal tract. Termed the gut microbiota, they have a collective mass of approximately 1.5 kg, and together with the gastrointestinal tract they create the most metabolically active system within the human body. Humans have coevolved with their microbial component over some two million years, leading to a homeostatic and symbiotic system designed for optimized production and absorption of essential nutrients, tightly regulated epithelial cell differentiation and renewal, and balance between immune recognition of pathogens and tolerance of commensal microorganisms. Only 10% of cells of this superorganism are represented by the Homo sapiens and millions of microbial genes present in the humans outnumber the "mere" 20–25 thousand genes of the human genome. The cultural, social, dietary, medical, and technological advances of the last two centuries tend to challenge this balance resulting in an alteration in the preexisting microbial composition and metabolic activity of the gut microbiome contributing to an unhealthy state. The scientific community is thus currently engaged in exploring approaches to manipulate the human gut microbiota by probiotic intervention as a means for disease prevention and treatment.

All this information about the gut microbiome and the possibility to manipulate it by probiotic therapy is highly exciting and full of possibilities. It is said that the next era therapeutics would involve maneuvering the resident microbiome of the human body to shift it from dysbiosis to symbiosis. It was in this context that 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 disease states, was perceived and planned. Each volume encompasses the potential and mechanism of probiotic therapy in a specific set of pathophysiology. With state-of-the-art commentaries on all aspects of probiotic research, from contributors across the globe, the e-book series provides an authoritative and timely overview of the field.

The first volume of the series comprising of 15 chapters, and expanding on the potential applications of probiotics in the management of cancer and immunological

diseases, is presented here. The introductory chapter discusses in detail the association of the gut microbiome with the suppression and progression of cancers. It also elaborates about the role played by the immune system in this interaction. Chapter 2 highlights the potential protective and therapeutic accountability of probiotics in combating cancer. The next chapter covers the role of various probiotic strains in cancer and summarizes the important findings in relation to the probiotic-mediated suppression of gastrointestinal and extraintestinal cancers. Chapter 4 elaborates on the future scope of probiotic therapy against a broad array of cancers like colon, stomach, breast, cervix, and myeloid leukemia cells, as an adjunct to chemotherapy or radiotherapy. In recent years, various researchers have highlighted the beneficial health effects of metabiotics, the components of probiotic microorganisms, and/or their metabolites. Potential of metabiotics as an effective strategy for prophylaxis or as a therapeutic option in the treatment of colorectal cancer is discussed in detail in Chap. 5. In the next five chapters (Chaps. 6, 7, 8, 9, and 10), the authors emphasize the role of probiotics in prophylaxis and management of various cancers, viz. colorectal, lung, gastrointestinal, and breast cancer. Efforts are made to include the underlying mechanism of action and a consolidated overview of the preclinical and clinical status of probiotic therapy in the management and control of these cancers. Next in line is the chapter highlighting the recent developments on applications of probiotic bacteriocins (ribosomally synthesized small antimicrobial peptides) along with other bacteriocins as anticancer agents, their cytotoxicity, efficacy, and mode of action against cancer cells. Chapter 12 encompasses the positive health effect of probiotics in autoimmune, inflammatory, and gastrointestinal disorders. It includes the underlying mechanism and the current market status of probiotics in the above-mentioned conditions. Elaborate discussion with respect to the status of probiotics in rheumatoid arthritis is presented in the next chapter.

An interesting perspective of genetically modified probiotics or designer probiotics is elaborated in Chap. 14. The sophisticated approach of using genetically engineered probiotic/designer probiotics is based on altering the genetic makeup of probiotic strains for improving human health, livestock management, and aquaculture industries. The chapter focuses on the current progress in the field of designer probiotics, safety concerns regarding their practical applications, and the potential prospects of their clinical translation. Last in the list is a chapter presenting a very elaborate glance on the patent world of probiotics to get the overall picture of the business potential of probiotics.

I hope this book will be a useful educational and scientific tool to academicians, health professionals, students, and pharma/biotech businessmen worldwide. As editors of the book, we express our sincere thanks to all the authors for their excellent contribution to the book.

Chandigarh, Punjab, India — Indu Pal Kaur
Ludhiana, Punjab, India — Parneet Kaur Deol

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About the Editors

Indu Pal Kaur is presently the Professor and Chairperson, at the University Institute of Pharmaceutical Sciences (UIPS), Panjab University, Chandigarh, India. She has more than 25 years of teaching and research experience to her credit. She was Director, Sophisticated Analytical Instrumentation Facility, Panjab University, from 2012 to 2015 and Dean, Faculty of Pharmaceutical Sciences, from 2017 to 2018.

Her research forte is enhancing the bioperformance of drugs, plant extracts/phytochemicals, and small/large biomolecules, viz. siRNA and probiotics using active-tailored delivery systems.

She has been granted seven Indian and one US patents and has filed 19 patents in the past 10 years. She has produced 23 PhDs and 55 postgraduates. She received funding to the tune of 72.3 million INR from government agencies and has a couple of industrial consultancies amounting to 15.3 million INR. Her research work was funded twice (2017 and 2020) by DST and Science and Technology Facilities Council, ISIS, UK for SANS characterization at ISIS Facility—Rutherford Appleton Laboratory, UK.

She has delivered 49 invited talks, published >130 high impact international publications, and her group has received 31 best paper awards at national and international conferences.

She is a US-Fulbright fellow (2017–2018) and was awarded Women Scientist Award 2018 by the Organisation of Pharmaceutical Producers of India (OPPI).

She has also been graced with BRIC Technology Exposition Award, consecutively for 2019 and 2020, Tynor Innovation Award 2019, and Researcher of the Year award 2019, by Sunpure Research Incubation Center.

The emphasis of her work lies in Industrial and clinical translation and is reflected through her three technology transfers to two Indian industries.

Parneet Kaur Deol is presently working as Assistant Professor at G.H.G. Khalsa College of Pharmacy Gurusar Sadhar Ludhiana, Punjab, India. She has more than 10 years of experience in probiotic research and has published her work in highly reputed peer-reviewed international journals. She has 21 international publications to her credit with a cumulative impact factor of >50. She has co-edited a special issue for "Current Pharmaceutical Design" with Prof. Indu Pal Kaur in the year 2019.

She has presented her work at various national and international platforms. She was awarded the "Dr. Harpal Singh Buttar and Mrs. Harinder Kaur Buttar Award of Excellence in Pharmaceutical Sciences" in the year 2016 and Mekaster Young Scientist Award in 2018 for her research work. Recently, she fetched two research projects from Department of Science and Technology-Science and Engineering Research Board (DST-SERB), New Delhi, worth 65 lakh.

Gut Microbiota and Cancer Correlates

1

Alok Malaviya, K. A. Paari, Shruti Malviya, Vamsi Krishna Kondapalli, Aditi Ghosh, and Riya Ann Samuel

Abstract

The human microbiota is a concoction of bacteria, archaea, fungi, and other microorganisms. It is necessary to maintain a partnership between the host and the microbiota in order to maintain the different aspects of human physiology, such as nutrient absorption, immune function and metabolism. The microbiota can contribute to both progression and suppression of the disease, including cancer. A disturbance in this interspecies balance called microbiome dysbiosis becomes a reason for the host to be more prone to issues such as immunodeficiency and cancer. Gut microbiota could potentially influence the factors that govern cancer susceptibility and progression through mechanisms such as immunomodulation, by producing metabolites, such as, bacteriocins, antimicrobial peptides involved in tumor suppression, and short-chain fatty acids (SCFA), and through enzymatic degradation. It is now an established fact that the host physiology as well as risk of diseases such as cancer could be greatly modulated by these commensal microbes and the regulation of cancer development, progression as well as response to anticancer therapy is greatly dependent on the host microbiota. Therefore, it is being envisaged that by the involvement of

A. Malaviya (✉) · V. K. Kondapalli · A. Ghosh
Applied and Industrial Biotechnology Laboratory (AIBL), Department of Life Sciences, CHRIST (Deemed to be University), Bengaluru, India
e-mail: alokkumar.malaviya@christuniversity.in; kondapalli.krishna@res.christuniversity.in; aditi.ghosh@science.christuniversity.in

K. A. Paari · R. A. Samuel
Department of Life Sciences, CHRIST (Deemed to be University), Bengaluru, India
e-mail: paari.ka@christuniversity.in; riya.samuel@res.christuniversity.in

S. Malviya
National Centre for Biological Sciences (NCBS), TATA Institute of Fundamental Research, Bengaluru, India
e-mail: shrutim@ncbs.res.in

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microbiome in augmenting antitumor responses to therapeutic approaches, potentially a new era of research with potentially broad implication on cancer treatment could be established. Better cancer treatment responsiveness can be achieved by understanding the role of the "tumor microbiome" in shaping the tumor microenvironment. This will help us to develop personalized anticancer solution with the goal to discover a bacterial species or a combination of species that decreases systemic toxicity and helps in anticancer therapy. This chapter is written in same context, which focuses on the association of the gut microbiome with the suppression and progression of cancers, the role of the immune system in this interaction, the utilization of these organisms for the treatment of cancers, and future perspectives.

Keywords

Bacteriocins · Cancer · Dysbiosis · Probiotics · Microbiome

1.1 Introduction

There are several different types of bacteria and other microorganisms present in the human body, which comprise the human microbiota. They inhabit in the epithelial barrier surfaces of the body exhibiting commensalism with the host. About 3×10^{13} bacterial cells are present in the gut microbiota whose composition is shaped by colonization at the time of birth, host genetics, type of delivery, incidences of diseases, exposure to antibiotics as well an individual's lifestyle. The composition of the microbiota changes during early years of life, which remains relatively constant throughout life. The gut microbiota impacts various aspects of human physiology, such as nutrient absorption, metabolism, and immune function. A continuous crosstalk between the gut microbiota, immune cells, and the mucosal barrier is necessary to maintain a healthy body (Roy and Trinchieri 2017). There are strong scientific indications that gut microbiota plays a shielding role against cancer in animal models and an imbalance of the gut microbiota (dysbiosis) might result in the development of many disorders, including cancer.

Probiotics are used to address the problem of gut microbiota imbalance, which when given in balanced quantity can provide health benefits to the host. They are known to have direct and indirect benefits on host well-being. While the direct benefit involves gut health improvement, these are also known to indirectly help with prevention and treatment of cancer, reduction in tumor formation and metastasis by modulating the microbiota, immune response, reduction in bacterial translocation, enhanced gut barrier function, and anti-inflammatory antipathogenic activity (Yu and Li 2016). Therefore, it has variously been suggested that probiotics could be used as a dietary supplement against neoplastic predisposition by influencing both the local and systemic immune processes of the host. It gives the hope that some probiotic strains can also be developed and used to prevent or treat cancers by functioning as adjuvants by modulating intestinal microbiota and immune responses. Owing to the ability of gut microbiota to modulate host metabolism, inflammation,

and immunity as well as its involvement in the initiation and/or progression of different cancers, this chapter is written with the aim to discuss various aspects of gut microbiota and gut dysbiosis and its association with cancer. Additionally, we have also discussed the various anticancer therapies based on gut microbiota.

1.2 Gut Microbiota, Gut Dysbiosis, and Cancer

Gut microbiota has a significant local as well as systemic effect on the nutrient absorption, metabolism, and immune function. Maintenance of the epithelial barriers is crucial for the health of the organism as it provides the surface for microbiota to reside on (Scott et al. 2013). The epithelial barrier and the commensal microorganisms maintain a peaceful relationship that mediates the protection of the host from pathogens and pathobionts. The physiological relationship between epithelial cells and the microbiota is disrupted by the alteration in the composition of the microbiota, a condition called dysbiosis (Fig. 1.1). Dysbiosis has been linked to the breach of the barriers, induction of inflammatory responses as well as initiation and progression of cancerous conditions (Roy and Trinchieri 2017). These organisms that comprise the microbiome are also believed to colonize tumors, and there are several models that suggest the role of the microbiome as a contributor to carcinogenesis. A healthy individual is said to be associated with high diversity of gut microbiota, which critically influences bacterial dysbiosis, pathogenesis, genotoxin production, and host metabolism disruption that controls the host immune system. Regulation of systemic function by the microbiota is crucial for the survival and health of the host (Yang et al. 2009). A lot of studies have been done to understand the metabolic functions of the associated microbes. However, the focus has shifted toward understanding the interconnections between physiologies of microbial communities, their host, and the impact of the gut microbiota to maintain health and disease (Hooper et al. 2007).

Decoding and sequencing of the microbiome have helped the researchers to get a clear sight of extending the benefits of manipulating the gut microbiota to treat diseases. Whole-genome shotgun sequencing and 16S ribosomal RNA amplicon sequencing help to deduce the diversity of particular taxa present in the gut microbiome. The information gathered will aid in reconstructing the potential metabolic capacity of the microbiome at strain, species, genus, and taxonomic levels (Saus et al. 2019). Advancement in metagenomic analyses has provided more direction to differentiate the gut microbiota present in diseased and healthy individuals. The past two or three decades have provided a sizable functional data relating to the presence of gut microbes in numerous physiological processes, including digestion of food substances and maturation of the immune system (Qin et al. 2010; Wong et al. 2019; Sender et al. 2016). Imbalance of microbiota or an impaired microbiota can result in the development of cancer, disturbing the host physiological functions through the interference with the immune system. Modulation of cancer treatment can be done by certain factors like antibiotic ingestion, defined microbiome transplantation, and change in lifestyle (Raza et al. 2018). The mechanisms using which these organisms affect the systemic function are less

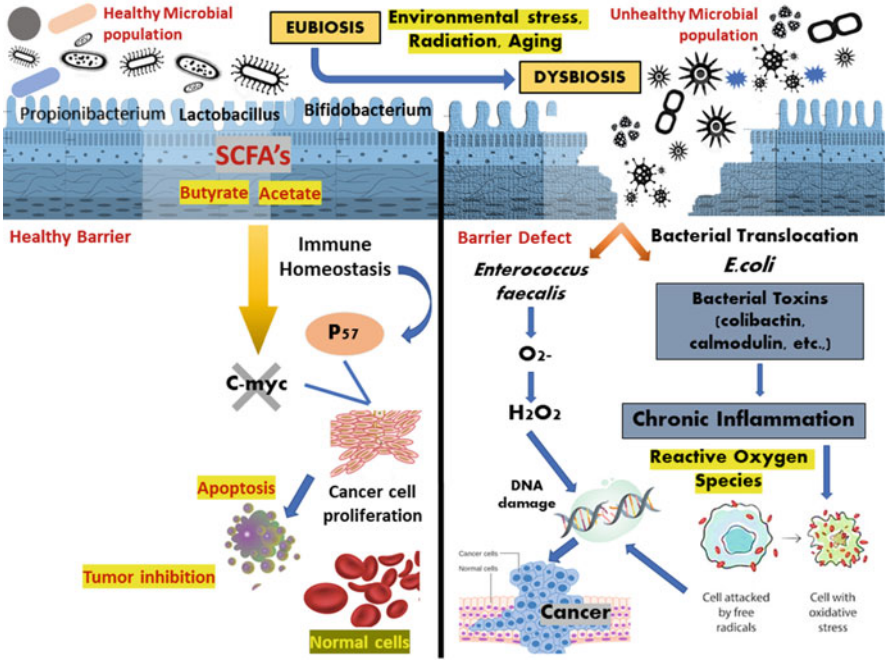

Fig. 1.1 Mechanism of gut microbiota in development and inhibition of carcinogenesis. Bacterial translocation happens because of imbalance in bacterial diversity (dysbiosis) causing chronic inflammation, resulting in the overexpression of proinflammatory cytokines and generation of reactive oxygen species causing oxidative stress and DNA damage resulting in carcinogenesis. In the presence of healthy microbial community (eubiosis), short-chain fatty acids (SCFAs) such as acetate, butyrate, and propionate are secreted, which create an immune homeostasis state influencing the process of cancer cell attenuation by limiting c-myc expression and by regulating P57 levels

understood when compared to the localized functions (Belkaid and Naik 2013). Following section briefly deals with an overview of gut microbiota and gut dysbiosis and its role in cancer.

1.2.1 Gut Microbiota and Its Interaction with Host

The human gut is a concoction of different bacteria, archaea and protozoa, which collectively constitute the microbiota (Gharaibeh and Jobin 2019). Constant crosstalk between these microbes, the mucosal barrier, and the immune system results in an efficient gut epithelial barrier (Ma et al. 2019). The central nervous system (CNS) and the "gut brain axis" (GBA) communicate and connect bidirectionally through the "gut brain axis" (GBA). Various components of GBA include (1) the autonomic nervous system (ANS), (2) the central nervous system (CNS), (3) the enteric nervous system (ENS), (4) the entero-endocrine system (EES), and

(5) the hypothalamic–pituitary–adrenal (HPA) axis (Vivarelli et al. 2019). Here the gut is a part of an interface between the resident microbiota of the gastrointestinal tract and the human body. In a bidirectional crosstalk between the human body and GBA, the gut microbiota acts as doorkeeper for such communications to happen (Neuman et al. 2015). It has variously been reported that the gut microbiome composition gets modified based on the host's hormones and neurohormones. For example, there are several peptide hormones secreted by the gastrointestinal entero-endocrine cells that could be sensed by the gut bacteria and in turn the gut microbiota composition is tuned. Similarly, gut microbiota also secretes some active molecules that are sensed by host's gut cells and translation of corresponding signals to GBA. It has been reported that gut microbiota can (1) produce vitamin K, vitamin B, and linoleic acid, (2) produce short-chain fatty acids, and (3) transform molecules such as glutamate to gamma-aminobutyric acid or histidine to histamine, affecting various aspects of the host health such as (1) modulation of host's immune system, (2) maintenance of host's gut barrier integrity, (3) modulation of host's metabolism, (4) xenobiotic and drug metabolism by host, and (5) host's protection against gastrointestinal pathogens (Vivarelli et al. 2019).

This partnership between the microbiota and the host is an essential element of health, and this interspecies balance is termed as eubiosis (Lazar et al. 2018). A disturbance in eubiosis termed microbiome dysbiosis is the alteration of the microbiota composition, which is associated with disrupting the microbiota–epithelial cell interaction. Switching of eubiosis to dysbiosis in the host becomes a reason for the host to be more prone to issues, such as immunodeficiency and cancer (Lazar et al. 2018, 2019). The role of gut microbiome in cancer is dual in nature as they play a role both in tumorigenesis and in the prevention and treatment of cancer. Cancer progression may alter the microbiome, and microbiome may also affect the progression of cancer. In diseases such as colorectal cancer, composition of bacteria in the host intestine was shown to be different in patients with colorectal cancer compared to healthy subjects (Gharaibeh and Jobin 2019). Intestinal epithelial cells function to provide mechanical protection and to regulate immunity by secreting chemokines, cytokines, and antimicrobial peptides (Wu and Wu 2012). Cytokines are soluble signaling proteins produced in immune cells such as macrophages, neutrophils, and B and T cells for the regulation of immune responses (Stenken and Poschenrieder 2015). They are also associated with maintaining a microbiota homeostasis. The cytokine interleukin-18 (IL-18) facilitates the protection of the intestinal mucosa, and mice deficient in IL-18 demonstrate dysbiosis that increases susceptibility to colon carcinogenesis (Roy and Trinchieri 2017). In a similar study carried out by Elinav et al. (2011), wild-type mice showed symptoms of dysbiosis after fecal microbiota of mice deficient in IL-18 were transferred to the wild-type mice.

1.2.2 Gut Microbiota as Cancer Promoter

The first bacterial protein to have been associated with human cancer is CagA produced by *Helicobacter pylori* (Vivarelli et al. 2019). *Fusobacterium nucleatum* (Fn), when present in abundance, has been associated with colorectal carcinoma

(Zhou et al. 2018). Fn contributes to colorectal cancer by using its FadA adhesin to bind to E-cadherin and causes the activation of host β-catenin–WNT signaling. Thus, FadA is a potential diagnostic and therapeutic target (Rubinstein et al. 2013). Certain bacterial pathogens make the host prone to cancer by promoting dysbiosis and altering the host's immune system and thus triggering the growth of tumor. Metalloproteinase toxin (MP toxin) by *Bacteroides fragilis* also plays a role by disrupting intercellular junctions and activating β-catenin signaling (Vivarelli et al. 2019). Bacteria such as *Bacteroides fragilis*, *Escherichia coli*, and *Peptostreptococcus anaerobius* have been associated with colorectal cancer through the activation of Th17 cell response and direct DNA damage (Wong et al. 2019). *Helicobacter hepaticus* has been reported to activate the WNT/β-catenin pathway as well as nuclear factor-kappa B (NF-κB)-regulated and Th-1 immune network resulting in hepatocellular carcinoma (Fox et al. 2010). Injuries to the epithelial barrier, inflammation, and chronic infections can trigger carcinogenesis in individuals. Infections due to various pathogenic microorganisms in the gut have been correlated with an increased risk of tumor development. Individuals with a *Salmonella typhi* infection are at the risk of developing gallbladder carcinoma; similarly, chronic *Streptococcus bovis* infection may lead to the development of colon cancer (Hooper et al. 2007). In the case of *H. pylori* and *S. typhi* infections, correlation between microorganisms and their tendency to initiate cancer in the hosts varies for different individuals (Mager 2006).

On the contrary, there are other organisms in the gut microbiota that are of great interest to the cancer researchers to mediate the effects of anticancer therapies (Wong et al. 2019). *H. pylori* has been reported to increase the risk of gastric cancer in some people and reduce the risk of esophageal cancer in others; however, the cause is still unclear (Whiteman et al. 2010). *Salmonella typhimurium* has been associated with gallbladder cancer, and it has also been used as a carrier of therapeutic agents for different types of cancers; as being a facultative anaerobe, it can easily survive in the anoxic environment often found in tumors (Mager 2006). They are made to migrate toward the tumor sites by rendering them auxotrophic for compounds found in high concentrations at the tumor sites such as by the removal of metabolic gene purI from mutants such as VNP20009. This forces the organism to move toward the tumor for survival (Low 2004). *S. typhimurium* destroys tumors by (1) using bacterial toxins to activate Caspase-3 for apoptosis, (2) delivering anticancer compounds, and (3) sensitizing the immune system to the tumors (Wall et al. 2010).

Several preclinical, clinical, and meta-analyses of clinical studies have explored the possibilities of manipulating the microbiota to change the host's response to different diseases, including cancer. One of the key mechanisms that scientists have tried to explore is immunomodulation (Ma et al. 2019). Immunomodulators change the way the immune system responds to the tumors by increasing (immunostimulators) or decreasing (immunosuppressive) antibody production (Bascones-Martinez et al. 2014). Recent studies have discovered that the bacteria in the gut impact the way cancer patients respond to immune checkpoint blockade therapy by using antibodies targeting co-inhibitory receptors to enhance the activity of T cell response (Gharaibeh and Jobin 2019)

1.2.3 Gut Microbiota as Cancer Suppressor

Several researchers have attempted to describe the mechanism by which gut microbiome influences the host physiology. The gut bacteria show anticancer effect either by increasing host immunity or by preventing gut dysbiosis. The bacteria *L. rhamnosus* GG (LGG) can counteract cancer growth in tumor models of ovarian, colorectal, breast, hepatic, cervical, and oral squamous cancers through its influence on mTOR or WNT pathways (Vivarelli et al. 2019; Nagy et al. 1998). One of the mechanisms involves short-chain fatty acids (SCFA), which are products of bacterial fermentation of undigested dietary fibers (Nagpal et al. 2018; Mager et al. 2005). They are associated with several functions such as intestinal repair, maintenance of intestinal homeostasis, inhibition of cancer cell proliferation, activation of G-protein-coupled receptors (GPCRs), etc. (Lazar et al. 2018; Nagpal et al. 2018; Arun et al. 2019). Butyrate and propionates show anticancer effect by inhibiting the histone deacetylases (Vivarelli et al. 2019; Ohland and Jobin 2015).

Probiotic bacteria such as lactic acid bacteria help the growth of SCFA-producing gut bacteria (Wang et al. 2019). Reduced production of SCFAs has been associated with an increase in the incidence of colorectal cancer (CRC) (Nagpal et al. 2018). Butyrate is an essential SCFA as it plays a key role in homeostasis (Lazar et al.2018). By inhibiting histone deacetylase (HDAC), it increases the acetylation of histone, which in turn regulates the transcriptional activity of tumor suppressors, resulting in a reduction of inflammation and CRC risk (Wang et al. 2019). *Escherichia coli* Nissle 1917 administered as mutaflor in combination with intestinal antibiotic rifaximin shows anti-inflammatory activity. Other probiotics such as *L. casei* trigger apoptosis and inhibit tumor growth by secreting a ferrichrome metabolite (Vivarelli et al. 2019). The association between microbiome and cancer is complex and has not been completely characterized. Additionally, various factors such as lifestyle, diet, and host immune system strongly influence the activity of the microbiota. Hence, it is difficult to conclude if their role is as promoters of cancer or as inhibitors.

1.2.4 Gut Microbiota as Immune Checkpoint Inhibitors

The gut microbiota helps the host immune system to develop tolerance toward beneficial microbiota and prompt an immune response against the gut pathogens as indicated in Fig. 1.2 (Vivarelli et al. 2019). The role of recognizing and attacking tumor cells is played by many cells of the immune system including T cells (Sharma and Allison 2015). In the presence of an antigen, T cells receive stimulatory signals for proliferation. T cells also receive inhibitory signals to downregulate their population once the infection is under control. Inhibitory signals can limit the response of T cells against cancer and hinder the process of tumor eradication (Andersen et al. 2006). CTLA-4 gene, programmed cell death protein 1 (PD-1) and its ligand (PD-L1) modulate down regulation of T cell response (Seidel et al. 2018). Thus, by targeting this inhibitory interaction, they can cause the T cells to remain activated for a period long enough for tumor eradication. The U.S. Food and Drug Administration

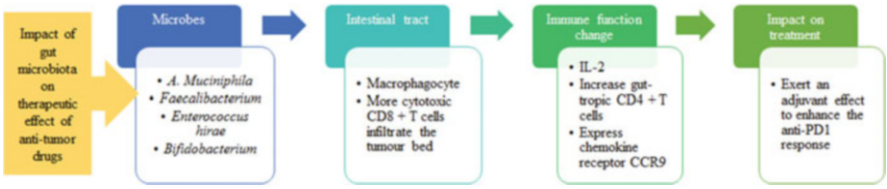

Fig. 1.2 Impact of intestinal microbiota on therapeutic effect of anticancer drugs by regulating immune system

(FDA) had approved antibodies such as ipilimumab, pembrolizumab, and nivolumab that target CTLA-4 and PD-L1, respectively (Sharma and Allison 2015). By targeting the inhibitory signals, the patient's own immune response is reactivated against the cancer. Although this strategy appears straightforward, the responses to these checkpoint inhibitors among patients are varied. Several human clinical studies have shown that the key players in influencing the checkpoint inhibitor response are the individuals' gut microbiota that have shown to affect the antitumor immunity and the efficacy of immunotherapy (Matson et al. 2018). Understanding the interaction between the microbiota and modulators of the immune system will pave the way to develop better therapeutic agents to treat cancer.

A study carried out in Jackson Laboratory (JAX) mice and the Taconic mice showed different rates of tumor growth and varied response to anti-PD-L1 antibodies due to the presence of *Bifidobacterium* sp. that has shown to possess antitumor activity by reactivating dendritic cells that in turn improve CD8-positive T cell against tumors (Sivan et al. 2015). When fecal matter from the mice that responded to treatment with anti-PD-L1 antibodies was transplanted into germ-free mice that would otherwise show no response to treatment, it was observed that there was an enhanced response to anti-PD-L1 therapy. The same was not observed when the fecal microbiota transplantation (FMT) was from mice that did not respond to therapy. This suggests the role of microbiota in the response (Gharaibeh and Jobin 2019). In a similar study carried out by Routy et al. (2018), *Akkermansia muciniphila* and *E. hirae* were found to be dominant in those patients responding to treatment with anti-PD-L1, while *Corynebacterium aurimucosum* and *Staphylococcus haemolyticus* were seen predominantly among patients who did not respond to treatment. *A. muciniphila* when given individually or when combined with *E. hirae* resulted in an improved response to PD-1 blockade in an IL-12-dependent manner by increasing the number of CCR9+, CXCR3+, and CD4+ T cells in the tumor beds (Routy et al. 2018). *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium* were also seen to be abundantly present in another cohort of patients of metastatic melanoma who responded to immunotherapy using anti PD-L1 (Matson et al. 2018). Several other studies provide evidence to support the fact that the gut microbiota is an important variable in cancer immunotherapy (Vétizou et al. 2015; Gopalakrishnan et al. 2018; Matson et al. 2018). While studying the role of the microbiota in patients with metastatic melanoma, Gopalakrishnan et al. (2018) found that the *Faecalibacterium prausnitzii* was in

abundance among the responders to anti-PD-1 therapy. These findings suggest that the composition of the microbiota possesses clinical significance for the treatment of cancer via immune checkpoint blockade therapy. Although the exact mechanism through which it is possible is not clear yet, manipulating these organisms may enhance patient's response to treatment (Gharaibeh and Jobin 2019)

1.2.5 Gut Microbiota, Inflammasomes, and Tumorigenesis

The gut microbiota makes use of multiprotein intracellular complexes called "*inflammasomes*" in order to interact with immune cells and gut cells (Vivarelli et al. 2019). They possess a subset of cytoplasmic pattern recognition receptors (PRRs) called NOD-like receptors (NLRs), using which they detect pathogenic and nonpathogenic microorganism-derived molecules and sterile stressors molecules. The inflammasomes are termed as the guardians of cellular and tissue integrity, as they are capable of playing an active role in responding to commensals and pathogens. Any imbalance in these complexes can result in a variety of diseases ranging from autoimmunity to cancer. These inflammasomes have been associated with both tumor promotion and suppression in different scenarios depending on the nature of the tumor and its microenvironment. The outcome of the inflammasome activation depends on factors such as (1) its expression pattern, (2) effector molecule, (3) tumor nature, (4) tumor stage, and (5) gut microbiome. While inflammasome-dependent IL-18 production plays an important role in suppressing colitis-associated CRC, inflammasome-dependent IL-1ß activation results in pro-inflammatory and tumor-promoting trigger resulting in the development of lung, skin, breast, and pancreatic cancer (Zaki et al. 2010; Salcedo et al. 2010).

Inflammasomal NLRs are a kind of innate receptors present in epithelial and innate immune cells, which aid in the detection of commensal microbiota and their bioproducts. These commensals and their bioproducts induce inflammasomal activation and IL-18 production in the gut, which helps in preventing intestinal barrier disruption and dysbiosis. Therefore, the hosts, which are deficient in inflammasomal components, are prone to reduced production of IL-18, leading to intestinal barrier impairment followed by larger penetration by commensal bacteria, increased inflammation, and finally trigger tumorigenesis (McLoed et al. 2016; Kolb et al. 2016; Daley et al. 2017). NLRs play a key role in regulating susceptibility to intestinal inflammation through its microbiome-modulatory activity (Vivarelli et al. 2019).

1.2.6 Gut Microbiota and Gastric Malignancies

Gastric cancer is a multifactorial disease affected by the environment, *H. pylori* infection, and other genetic factors. Gastric cancer is an inflammation-associated cancer (Meng et al. 2018). An infection with *H. pylori* triggers the initial steps of carcinogenesis through a decrease in acid production that allows other bacterial communities to grow, leading to increased inflammation and degradation of the epithelial barrier (Ferreira et al. 2018). Studies also suggest that along with inducing

inflammation these microbes interfere with anticancer agents (Meng et al. 2018). Although *H. pylori* is the lead player in gastric carcinomas, it cannot be described under gut microbiota, since it is a pathogen and not a commensal. The microbiome has its influence on the cancers of the GI tract including pancreatic, liver, colorectal, and gastric cancers (Meng et al. 2018). Several bacteria other than *H pylori* are also associated with carcinogenesis through mechanisms like (1) inflammation promotion, (2) modification of the action of stem cells, and (3) stimulation of cell proliferation and production of toxic metabolites (Petra et al. 2017). In contrast, study conducted to compare the microbiota of patients suffering from gastric cancer and normal patients revealed that there was no significant difference in compositions between the two suggesting that microbiota are just bystanders in the progression of cancer (Dicksved et al. 2009). A study carried out by Maldonado-Contreras et al. (2011) compared the gastric microbiota of *H. pylori* positive and negative individuals. The study showed that *H. pylori* positive individuals are having an increased count of *Acidobacteria*, *Proteobacteria*, and *Spirochaetes* and reduction in *Firmicutes*, *Bacteroidetes*, and *Actinobacteria*. Similarly, *H. pylori* negative individuals showed an increase in *Firmicutes, Bacteroidetes*, and *Actinobacteria* (Maldonado-Contreras et al. 2011; Bik et al. 2006). Difference in the organisms associated with esophageal cancer and a healthy esophagus was noted. Firmicutes, Proteobacteria, and Bacteroidetes were the common phyla in samples of esophageal squamous cell carcinoma (ESCC) and esophageal squamous dysplasia (ESD) patients indicating their association with tumorigenic process (Yang et al. 2012; Aghazadeh et al. 2017; Nasrollahzadeh et al. 2015).

Colorectal cancer (CRC) is one of the leading causes of cancer mortality in the world (Fleming et al. 2012; Sasaki et al. 2005). Exposure of microorganism is continuous from the mouth to the anus mostly because the gastrointestinal epithelium is connected to the environment. One of the most preferable study sites for microbial diversity is colon as bacterial load is more and it follows the hierarchy of jejunum, duodenum, and least is at the oral cavity. Understanding of the recent advancement in the microbiome study has established a huge impact on human health. The association between the microbial abundance and cancer incidence has indicated the significant role of microbiota in colorectal cancer (Wong et al. 2019).

Experimental, geographical variation, and migration studies have provided compelling evidence that both environmental and genetic alteration is the reason for the formation of CRC. Development of CRC is basically due to the epigenetic modification of several genes and accumulation of mutation. According to the definition provided by Louis et al. (2014), CRC mostly occurred by the transition of normal mucosa to premalignant lesions due to sequential genetic alteration and mutation. The reason thought to drive this mutation is by the origin of mutation in the adenomatous polyposis coli tumor suppressor gene, which encodes a protein that plays a significant role in WNT pathway, intercellular adhesion, regulation of the cell cycle, and apoptosis (Louis et al. 2014). Another risk that is specified for the commencement of the CRC is the association with diet and lifestyle. Epidemiological studies have pointed out that consumption of excessive protein and fat from red and processed meat can escalate the risk of development of colorectal tumorigenesis

(Yang and Yu 2018). The colonic health is maintained by residues of diet such as complex carbohydrates, protein residues, and primary bile acids, which are absorbed in the intestine. Hence ensuring a balanced diet can critically protect from the risks of CRC as the saccharolytic fermentation of complex carbohydrate will produce short-chain fatty acids (SCFAs) and butyrate, which pose anti-inflammatory and antineoplastic properties through the acceleration of cellular metabolism, microbiota homeostasis, antiproliferation, immunomodulatory, and genetic and epigenetic regulation (O'Keefe 2016).

Clinical and epidemiological studies show the mechanism of gut microbiota interaction and its vital relationship to human health. Administering adequate amount of probiotics (live organisms) is of much interest for researchers as supplementation of probiotics in the right amount would improve the ecological health of microbiota, which can convert dysbiosis to eubiosis and can be an alternative option for treatment of antibiotics (Neish 2009). Many researchers have suggested the significance of probiotics and its administration as an alternative. Bacteria like Proteobacteria, Verrucomicrobia, Actinobacteria, Fusobacteria, and Cyanobacteria help in metabolizing complex carbohydrates into short-chain fatty acids, which increase apoptosis in the colon (Singh et al. 1997; Berdanier 2018). As the large intestine comprises the majority of the microbial wealth, researchers are keen in understanding the intestinal environment and the abundance of the type of bacteria that contribute to carcinogenesis and/or tumor protection. Interaction of tumor with its local microenvironment and its systemic effects on the host revealed the imbalance and destruction of gut microbiota (Lin et al. 2019). According to Lin et al. (2019), *Fusobacterium nucleatum*, *E. coli*, *Bacteroides fragilis*, and *Peptostreptococcus anaerobius* are the bacteria that were abundant in the tumor tissues of the patients, which have the ability to promote CRC.

Lactobacillus casei BL23 has shown to increase the apoptosis rate by inducing the production of Caspase-9, Caspase-7, and Bik that help in inhibiting the cell proliferation of CRC (Jacouton et al. 2017; Yang and Yu 2018; Lenoir et al. 2016). *Lactobacillus pentosus* B281 and *Lactobacillus plantarum* B282 have been reported for their ability to arrest the G1 phase of the cell cycle resulting in the downregulation of certain cyclin genes, thereby inhibiting the growth of colon cancer (Saxami et al. 2016). *Pediococcus pentosaceus* GS4 (Dubey et al. 2016), *Lactobacillus* BCRC1710 (Saber et al. 2017; Nekouian et al. 2017), *L. acidophilus* CL1285, *L. casei* LBC80R, and *L. rhamnosus* CLR2 are other few bacterial strains that have been found to control colorectal cancer (Desrouillères et al. 2015). LAB strains prevent the formation of aberrant crypt foci, a precursor of colorectal cancer by the induction of hepatic detoxifying enzymes and by inducing apoptotic proteins Bax/Bcl-2 causing significant damage to the cancer cells. *Pediococcus* controls proliferation of the tumor cells that cause colon cancer by triggering apoptosis by downregulating NF-kB and p-Akt. Evidence suggests that several LAB strains possess antioxidant activity that can reduce the oxygen-centered free radicals in the gastrointestinal tract (Yeh et al. 2013; Kanmani et al. 2013). *L. salivarius*, which has been isolated from fecal samples, has been reported to suppress 4NQO-related spread of cancer in rats by decreasing induced apoptosis and the expression of

proliferating cell nuclear antigen in a dose-dependent style (Zhang et al. 2013). Yeh et al. (2013) have reported an improvement in the serum albumin, prealbumin, and body weight of patients treated with cancer medicine and were given nutritional supplements containing probiotics (Yeh et al. 2013). *Bifidobacteria*, which produces butyric acid, could initiate apoptosis in the colon and also inhibit *2-amino-3-methylimidazo*-induced colon, liver, and mammary carcinogenesis (Reddy 1997).

Fermentation of dietary nondigestible carbohydrates is the source of energy for gut microbiota. SCFAs, such as propionate, butyrate, and acetate, exhibit anti-inflammatory properties in human monocytes, whereas butyrate constitutes a major energy source for colonocytes (Neish 2009). Inulin-type fructans reduce the growth of hepatic BaF3 cells and lessens the inflammation by a cAMP level-dependent pathway. The gut microbiota has influenced BaF3 cell progression by varying its metabolome (Bindels et al. 2012). Gut microbiota improves the potential of manipulating the efficiency of treatment for cancer by reducing the side effects (Qin et al. 2010). Proteolytic enzymes of lactic acid bacteria help in cleaving the milk protein during fermentation, and the resulting biological peptides can trigger apoptosis activation and inhibition of cancer cells via cell membrane disruption without having a negative effect on the healthy cells. The reports of a study suggested that use of four proteolytic strains of *L. helveticus* grown under the skim milk has the capability of releasing bioactive compounds (Elfahri et al. 2016). However far too little attention has been paid toward the etiologic role of chronic infections in carcinogenesis. Diversity of pathogens extracted from the tissue samples of lung cancer patients has pointed out that mycoplasma strains were spotted in all samples; more often the strains of *Staphylococcus epidermidis*, *Streptococcus mitis*, and bacterial strains like candida, listeria, and chlamydia have shown a descending frequency (Apostolou et al. 2011). *L. casei* SR4 and *L. paracasei* SR2 strains show anticancer activity by the upregulation of BAX, BAD, Caspase-3, Caspase-8, and Caspase-9 genes and by downregulating the Bcl-2 gene, hence preventing cervical cancer. *Streptococcus thermophilus* M17PTZA496 and *Streptococcus thermophilus* TH982 act against cancer cells by releasing folic acid, histamine, and tyramine possessing high cytotoxic effects against cancer cells (Tarrah et al. 2018). Secretions of *Kluyveromyces marxianus* AS41 can cause the activation of extrinsic and intrinsic pathways for cancer cell apoptosis by upregulation of BAD, Fas R, CASP 9, CASP 8, and CASP 3 (Saber et al. 2017).

1.3 Gut Microbiota and Anticancer Therapies

The final goal of anticancer therapies is to be efficient in eradicating targeted malignancies. Radiotherapy, chemotherapy, and immunotherapy treatments are the pillars of the currently applied cancer treatment system, and almost all of these available treatments have detrimental effects toward normal cells including the normal gut microbiota leading to gut dysbiosis. Eventually, such altered microbiome composition can significantly affect the patient's response toward applied anticancer therapies (Roy and Trinchieri 2017; Vivarelli et al. 2019). Therefore, novel strategies

to manipulate the gut microbiome needs to be evaluated and identified so as to maintain the intact gut microbiome to finally improve the patient's therapeutic outcome. In the following section, we will discuss various aspects of gut microbiota in relation to their role and application in various anticancer therapies.

1.3.1 The Tumor Microbiome and Its Application in Anticancer Treatment

Several studies have demonstrated that certain microorganisms preferentially colonize and replicate in the tumor microenvironment. Presence of such microbes in tumor tissue may be a direct cause for tumorigenesis, as seen in the case of *H. pylori* colonization in gastric cancer. Pockets of necrosis and hypoxia due to insufficient blood supply and increased oxygen demand from rapidly growing tumor cells along with immune suppressed microenvironment formed within the tumor niche provide favorable environment for proliferation of certain bacterial communities (Bashirdas et al. 2017). This could result from coinciding infection or bacterial translocation from the gut lumen due to epithelial barrier disruption. This local colonization of bacterial communities in tumor environments is termed as "tumor microbiome," and various reports have suggested a complex interaction between the microbiome of the tumor and tumor immunity (Rubinstein et al. 2013; Gur et al. 2015). Tumor microbiome may play roles in the (1) development of tumor resident microbes and (2) reduction of treatment-related systemic adverse effects by distribution of therapeutics specifically at tumor sites (Bashirdas et al. 2017).

There are various antitumor immune stimulatory effects that could be mediated by structural components of tumor-associated bacteria such as flagellin, peptidoglycan, LPS, and other pathogen-associated molecular patterns (PAMs), which could be applied to elicit antitumor immune response. Additionally, affinity of anaerobes such as clostridial spores to germinate in hypoxic regions of solid tumors is also being experimented as a therapeutic approach. Attenuated clostridial spores have been tested in canine tumors (Roberts et al. 2014). Similarly, attenuated *Salmonella* strain has also been utilized as agents against cancer due to their ability to colonize tumors. The antitumor activity of these attenuated bacterial strains is known to be facilitated by bacteremia-induced TNF-alpha secretion. Due to the vasoactive property of these secreted products, they facilitate the entry of bacteria into the tumor microenvironment, which results in activation of $CD8^+$ T cell numbers for improved tumor surveillance and clearance (Leschner et al. 2009; Stern et al. 2015; Bashirdas et al. 2017).

The nonspecific toxicity of systemically administered therapeutic agents has been reported to be reduced by using bacteria as tumor-specific targeted drug delivery platforms. Various tumor-targeting agents such as bacterial toxins, cytokines, and immune activating proteins have been designed for specific delivery at tumor sites using bacterial vehicles (Bashirdas et al. 2017). Similarly, a quorum sensing-based interbacterial communication system has been applied for targeting regions that are hypoxic and inaccessible to chemotherapy agents (Ryan et al. 2009). These

approaches are elegant and optimistic, which require further finetuning and optimization to make them effective.

1.3.2 Gut Microbiota and Modulation of Chemotherapy and Immunotherapy Efficiency

Cancer pathogenesis along with its therapeutic outcome could be significantly impacted by dysbiosis in the composition of gut microbiota, as the gut microbiota has the ability to (1) metabolize antitumoral compounds, (2) modulate the immune response of host, and (3) modulate inflammation pathway (Vivarelli et al. 2019). With reference to chemotherapy, it has been observed that efficacy of certain anticancer drugs, e.g., cisplatin and cyclophosphamide, was altered depending on the presence or absence of fully functional gut microbiota or coupling the treatment with certain probiotic strains such as *L. johnsonii* and *E. hirae* (Iida et al. 2013; Gui et al. 2015; Viaud et al. 2013; Daillère et al. 2016). Similar effects have been observed with respect to immunotherapeutic treatments when administration of CpG oligodeoxynucleotide (immunotherapeutic agent that is synthetic molecule mimicking bacterial DNA) along with *Alistipes shahii* resulted in improved immunotherapeutic outcome as compared to the condition where CpG was administered alone (Iida et al. 2013).

Patient's gut microbiome composition has also been linked to the intrinsic efficacy of immune checkpoint inhibitor-based immunotherapy, where the immune inhibitory pathway was blocked by use of therapeutic agents so as to modulate the T cell activation against tumor target cells (Vivarelli et al. 2019). It was observed that enrichment of gut microbiome with *Bacteroides fragilis* and *Burkholderia cepacia* significantly increased the efficacy of anticytotoxic T lymphocyte-associated protein 4 (CTLA4) antibodies, which were used for reducing sarcoma tumor growth in mice. Similarly, administration of *Bifidobacterium* sp. was found to improve the efficacy of programmed death ligand 1 (PD-L1) targeting antibody in mice model (Vétizou et al. 2015; Sivan et al. 2015). In line with these reports, Gopalakrishnan et al. (2018) has demonstrated that the microbiome of anti-PD-L1 responders is significantly different from nonresponders. Similarly, Matson et al. (2018) reported the significance of *E. faecium*, *Bifidobacterium longum*, and *Collinsella aerofaciens* in ameliorating the anti-PD-L1 efficacy (Matson et al. 2018). PD-L1 therapy is done to generate the antitumor immunity, which works by prevention of the interaction between PD1 protein and PD-L1. It has been found that the prevention of this interaction is enhanced by the various components of gut microbiota. Therefore, it was concluded that the bacterial immune synergy for response to anti-PD-L1 therapy is facilitated by intestinal microbial communities (Sivan et al. 2015; Roy and Trinchieri 2017; Jobin 2018). Contrary to this, there are specific microorganisms whose presence, in vicinity or even at a distant site, can interfere with the treatment of cancer. For example, presence of *Escherichia coli* (Enterobacteriaceae) strains have been reported to negatively interfere with tumor response against the chemotherapeutic agent gemcitabine, whose efficacy was compromised by metabolization

or deactivation of the active form of the drug (Jobin 2018). Another example is that of Firmicutes such as *Faecalibacterium*, which when present in increased numbers can result in toxic side effects after anti-CTLA4 antibody treatment. A decrease in the abundance of Bacteroides also has a similar effect. Similarly, it was found that the introduction of *Akkermansia muciniphila* reversed the low response to PD-1 blockade in mice receiving human nonresponder FMT. This showed an improvement in antitumor immune cell infiltration and activity in tumors indicating that these microbes can be used to improve the precision of cancer medicines (Jobin 2018).

1.3.3 Use of Probiotics in Cancer Treatment

Because of their ability to preserve gut homeostasis, probiotics are tested against gut dysbiosis in cancer patients undergoing chemotherapy and radiotherapy (Table 1.1). Probiotics work locally as well systemically and exert their antitumor properties by a combination of events such as (1) antioxidant activity improvement, (2) host's immune response modulation that includes both gut associated and systemic immune responses, (3) improvement of gut homeostasis and bacterial translocation, (4) carcinogen degradation, etc. (Reid et al. 2003; Yu and Li 2016).

Probiotic lactobacilli have variously been reported to significantly reduce the prevalence of colon cancer. It was found that the administration of these probiotic bacteria leads to modification of the enteric flora of mice and by influencing the overgrowth of bacteria and their translocation in Wistar rats after 80% gut resection (Yu and Li 2016) (Table 1.2). In one such study conducted by Konishi et al. (2016), it was found that ferrichrome produced by *L. casei* ATCC334 acts as a tumor-suppressive molecule, responsible for its observed tumor-suppressive effect. This molecule when used on colon cancer cells showed a strong tumor-suppressive effect by activating c-jun N-terminal kinase (JNK) signaling pathway. Another study showed a positive impact on colon cancer reduction by lowering the activity of certain enzymes when the organism *L. rhamnosus* LC705 was used in combination

Table 1.1 Antitumoral effects of gut microbiota and corresponding effectors

Microorganisms	Effectors	Effects
E. coli	Colibactin; CDT	DNA double-strand breaks
S. flexneri	IpgD; VirA	PS3 degradation
H. pylori	CagA	PS3 degradation; catenin; MAPK; AKT pathway activation; ROS production
F. nucleatum	FadA	β-catenin pathway activation
B. fragilis	MP toxin	β-catenin pathway activation
S. enterica	AvrA	β-catenin, MAPK and AKT pathways activation
F. nucleatum	Fap2	Blockage of antitumor immune response
E. faecalis	Superoxide	ROS production
C. leptum, *C. coccoides*	β-gluc	Estrogen receptor activation

Table 1.2 List of probiotic strains used for evaluation of their anticancer effects

Probiotic strain	Type of cancer	Mechanism of action
Lactobacillus casei BL23	Colorectal cancer	Inhibition of cell proliferation (Hooper et al. 2007)
Lactobacillus pentosus B281 and *Lactobacillus plantarum* B282	Colon cancer	Antiproliferative activity (Hooper et al. 2007)
Bacillus polyfermenticus KU3	HeLa, LoVo, HT-29, and MCF-7cancer cell lines	It showed negative impacts on the proliferation of different cancer cell lines (Scott et al. 2013; Lee et al. 2015)
Lactococcus lactis	Human breast adenocarcinoma cell line	Process cytotoxic effect on MCF-7 cells (Yang and Yu 2018)
Lactobacillus casei SR2 and *Lactobacillus paracasei* SR4	Cervix cancer (Hela) cell line	Antitumor activity (Qin et al. 2010; Chondrou et al. 2018)
Lactobacillus acidophilus CL1285, *Lactobacillus casei* LBC80R, and *Lactobacillus rhamnosus* CLR2	Colon cancer	Prevention of aberrant crypt foci (ACF) formation (Wong et al. 2019; Riaz Rajoka et al. 2018)
Streptococcus thermophilus M17PTZA496 and *Streptococcus thermophilus* TH982	Cancer cells	Anticancer activity (Sender et al. 2016)
Lactobacillus acidophilus and *Bifidobacterium bifidum*	Hepato cellular carcinoma cancer	Upregulation of tumor suppressor gene and downregulation of Bcl-w and KRAS (Raza et al. 2018; Heydari et al. 2019)
Lactobacillus reuteri	Gastric cancer	Reduced the proliferation of cancer cells, and uPA and uPAR gene expressions are attenuated, which are involved in cancer metastasis (Belkaid and Naik 2013)
Lactobacillus salivarius	Oral cancer	Downregulation of proliferating cell nuclear antigen and induced apoptosis in a dose-dependent manner (Saus et al. 2019)
Bifidobacteria	Colon cancer	Creation of bactericidal environment for putative enteropathogens such as *E. coli* and *C. perfringens* (Fleming et al. 2012)
Mariprofundus ferrooxydans	Cancer cells	Blocking metastasis (Louis et al. 2014; Kumeria et al. 2016)

with *Propionibacterium freudenreichii* sp. Shermanii JS (Hatakka et al. 2008; Brady et al. 2000). Similarly, probiotic yogurt has been found to be effective in controlling *Bacteroides fragilis* (ETBF) associated with inflammatory bowel disease and colorectal cancer (Odamaki et al. 2012).

Probiotics positively influence intestinal health by protecting the intestinal barrier and by minimizing the DNA damage in intestinal tissues. During the initial stages of colorectal cancer, there is a disruption of the tight junctions that causes loss of integrity across the intestinal barrier. It has been shown that the introduction of certain prebiotic and probiotic (*Lactobacillus plantarum*) can prevent the disruption of the epithelial barrier (Commane et al. 2005; Ko 2007). Similarly, permeability across intestinal barrier as well as attenuation of inflammatory response was reported by the administration of certain probiotics in patients undergoing biliary drainage (Jones et al. 2013). Similarly, DNA damage or adduct formation by mutagen was found to be reduced by administration of probiotic molecules (Horie et al. 2003; Yu and Li 2016). In this context, it has been reported that similar to the tumor-suppressor protein p53, probiotics exert their functions by channelizing the cell apoptosis during elevated DNA damage levels (Zhang et al. 2009).

It has been reported that daily intake of *L. casei* has a positive effect on natural killer (NK) cell activity (Takeda and Okumura 2007). Similarly, in animal models a decrease in the occurrence of colon cancer was observed through immunomodulatory effects of probiotics and/or synbiotic administration (Yu and Li 2016). Enhanced NK cell number or cell cytotoxicity, CD4/CD8-positive lymphocytes, or phagocytic activity of macrophages were found in rats or mice treated with probiotic products (Roller et al. 2004; de Moreno de LeBlanc et al. 2005; Yu and Li 2016; Guha et al. 2019). *B. lactis* sp 420 when tested on human colon carcinoma cell line Caco-2, it was found to exert anti-inflammatory and anticarcinogenic properties by modulating cyclooxygenase expression profile (Nurmi et al. 2005). Probiotics exert their anti-inflammatory effect by regulation of inflammatory mediators such as interferons, interleukins, and cytokines. Regulation of anti-inflammatory activity results in beneficial effects such as effective control of inflammation and carcinogenesis. Improving the functioning of antioxidative enzymes has also been reported to be exerted by probiotics, which is known to help against carcinogen-induced damage (Yu and Li 2016). A list of bacterial products and their anticancer mechanism has been presented in Table 1.3. Hence, it could be concluded that probiotics developed based on gut-microbiota could be developed as a potential anticancer therapy.

The problem of chemotherapy-associated gastrointestinal toxicity has also been addressed by use of probiotics (*Lactobacillus* spp.) as supportive treatment strategy, pertaining to their anti-inflammatory activity within the intestinal microenvironment. *L. rhamnosus* GG (LGG) is one of the first studied probiotic model species used in cancer-related studies (Chen et al. 2017). Several clinical trials have also attempted to study the role of LGG administration in order to prevent the toxic effects of anticancer therapies as well as its potential role in the direct modulation of cancer development (Tables 1.4 and 1.5) (Vivarelli et al. 2019).

Table 1.3 Different bacteria products and their anticancer mechanism

Strain	Product	Influence	References
Lactobacillus rhamnosus	SCFAs	Influences mTOR or WNT pathway Counteracts cancer growth	Vivarelli et al. (2019)
Lactic acid bacteria	SCFAs—butyrate and propionates	Inhibition of histone deacetylases Increases acetylation Decreases transcriptional activity of tumor suppressors Decreases inflammation and CRC risk	Wang et al. (2019)
Lactobacillus casei	Ferrichrome metabolite	Increases apoptosis Decreases tumor growth	Vivarelli et al. (2019)
Pseudomonas sp.	Azurin	Inhibition of cell signaling Inhibition of angiogenesis Stabilization of p53	Sadhu and Ganguly (2017)
L. acidophilus	Polysaccharide fraction	Induces apoptosis	Sadhu and Ganguly (2017)
L. acidophilus	Polysaccharide	Regulates the expression of BCl-2 interacting protein and cell division cycle protein	Sadhu and Ganguly (2017)
E. coli	Colicin	Generates pores in the plasma membrane Activates apoptosis	Kaur and Kaur (2015)
Klebsiella pneumonia RYC492	Microcin E492 (M-E492)	Cell shrinkage DNA fragmentation and extracellular exposure of phosphatidylserine Activation of caspases Loss of mitochondrial membrane potential Release of calcium ions from intracellular stores Apoptosis of cancer cells	Kaur and Kaur (2015)
Lactobacillus sp.	Nisin	Binding of bacteriocin proteins to lipid II Prevents the transport of peptidoglycan subunits to the cell wall Synthesis of incorrect cell wall Cell death (Or) Pore formation and membrane insertion Cell death	Kaur and Kaur (2015; Todorov et al. 2019)
Bacillus spp.	Mersacidin	Interferes with cellular enzymatic reactions cell wall synthesis	Kaur and Kaur (2015)
Lactococcus lactis subsp. *lactis*	Lacticin	Pore formation in the cell membrane Depolarization of the cytoplasmic membrane	Kaur and Kaur (2015)

Table 1.4 Various completed clinical trials to establish the role of probiotics in cancer patients

Title and (Clinical Trials Gov identifier)	Intervention	Disease
Probiotics in CRC patients (NCT00936572)	DS: probiotic *La1*	CRC
Impact of probiotics on diarrhea in patients treated with pelvic radiation (NCT01839721)	DS: probiotic *Bifilact*	Various cancers
Prevention of irinotecan-induced diarrhea by probiotics (NCT01410955)	DS: probiotic *Colon Dophilus*	CRC
Synbiotics and GI function-related quality of life after colectomy for cancer (NCT01479907)	DS: prebiotics and probiotics *Synbiotic Forte*	CRC
Impact of probiotics on the intestinal microbiota (NCT01609660)	DS: *S. boulardii*	CC
Using probiotics to reactivate tumor-suppressor genes in CRC (NCT03072641)	DS: probiotic *ProBion Clinica*	PC
Effect of probiotics in patients undergoing surgery for periampullary neoplasms (NCT0.1468779)	DS: probiotics	CRC
Impact of probiotics in modulation of intestinal microbiota (NCT01895530)	DS: *S. boulardii*	CRC
Action of synbiotics on irradiated GI mucosa in RC treatment (FIPIREX) (NCT03420443)	DS: probiotics	RC
Intestinal microbiota in lung cancer after chemotherapy (NCT02771470)	DS: probiotics	LC
Influence of probiotics administration before liver resection in liver disease (LIPROCES) (NCT02021253)	DS: probiotics	HCC

Table 1.5 Various clinical trials to establish the impact of fecal microbiota transplantation (FMT) in cancer treatment

Title and (Clinical Trials Gov identifier)	Intervention	Disease
Safety to stool transplant for patients with difficult to treat *C. difficile* infection (NCT02770326)	FMT	Various cancer
Prevention of dysbiosis complications with autologous FMT in acute myeloid leukemia patients undergoing intensive treatment (NCT02928523)	Autologous FMT	Acute myeloid leukemia
FMT in metastatic melanoma patients who failed immunotherapy (NCT03353402)	FMT	Melanoma
FMT in melanoma patients (NCT03341143)	FMT with Pembrolizumab	Melanoma

1.3.4 Use of Fecal Microbiota Transplantation (FMT) in Cancer Treatment

Fecal microbiota transplantation (FMT) has variously been projected as an alternate strategy used to cure pathogen infection or in treatment of gut diseases, e.g., recurrent *Clostridium difficile* duodenal infection has been cured by FMT (van Nood et al. 2013). Similarly, the efficacy of FMT in reducing colon tumorigenesis has been seen during preclinical studies done in mice. Clinical trials are in progress

to evaluate and establish the use of FMT in treatment of cancer as well as in preventing the intestinal side effects of anticancer treatment (Vivarelli et al. 2019) (Table 1.5). Once established, FMT could be developed as an efficient antitumor therapeutic strategy.

1.4 Conclusion and Future Prospect

The gut microbiome and the respective host share a complex relationship among themselves. The gut microbiota is inherited by people and changes depending on factors such as age, diet, and environment. This microbiota footprint changes during the lifetime of each individual. The gut microbiota has been studied in great detail for its performance with respect to a number of important functions, such as protection from infections, pathogen colonization control, dietary compound hydrolysis, and vitamin production. It is now an established fact that the host physiology as well as risk of diseases, such as cancer, could be greatly modulated by these commensal microbes. Regulation of cancer development, progression as well as response to anticancer therapy is greatly dependent on the host microbiota. And therefore, a potentially new era of research with potential broad implication on cancer treatment could be envisaged by the involvement of microbiome in augmenting antitumor responses to therapeutic approaches. Better cancer treatment responsiveness can be achieved by understanding the role of the "tumor microbiome" in shaping the tumor microenvironment. Researchers are considering personalized cancer treatment by modifying the patient's microbiota as a possibility. The individuals' microbiota composition could be used as a biomarker, a diagnostic tool, and possibly a therapeutic target due to its resilience, stability, and responsiveness to environmental, physiological, and pathological changes (Lee et al. 2017). This will help us to develop personalized anticancer solutions with the ultimate goal to discover a bacterial species or a combination of species that decreases systemic toxicity and helps in anticancer therapy. To make it a success, we need to apply modern scientific advancements for microbiome-based patient stratification rather than relying on population-based data or frequently used "trial-and-error" approaches. In this direction, modern advancement in data sciences like artificial intelligence and machine learning approaches may enable us to tailor treatment combinations so as to more optimally achieve therapeutic efficiency while minimizing adverse effects. Overall, this approach represents a new and exciting frontier toward future harnessing of microbiome as a diagnostic tool (Bashirdas et al. 2017).

Therefore, it is expected that targeting the microbiota is likely to become one of the next frontiers for personalized medicine (Roy and Trinchieri 2017). Targeted interventions on microbiome by supplementation of prebiotic and/or probiotic might be used as preventive healthcare solutions for cancer as well as to improve the efficacy of the existing cancer treatments such as chemotherapy, radiotherapy, and immunotherapy.

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Potential Preventive and Therapeutic Accountability of Probiotics in Cancer: An Insight of Mechanism of Action

2

Pranav Kumar Prabhakar, Yachana Mishra, and Vijay Mishra

Abstract

Probiotics, the living microorganisms such as bacteria and yeast, are used for the treatment of various disorders like allergies, diarrhoea, vaginosis, inflammatory and irritable bowel disease, lactose intolerance, diabetes and cancer. In recent time, anticancer properties of probiotics have been extensively studied. Some probiotics reduce the proliferation and growth of the microbes, which produce the mutagens and carcinogens, modify the metabolism of carcinogens, protect DNA from the oxidative damage and free radicals effects, provide competitive adherence to the mucosa and epithelium, produce antimicrobial substances and lastly manage the immune system. Various scientific reports with cell line, animal studies and human studies have advocated the beneficial therapeutic effects of probiotics in the suppression of cancer cell metastasis and invasiveness both in vivo and in vitro. However, more precise pre-clinical and clinical studies are warranted to establish the therapeutic potential of probiotics in cancer therapy.

Keywords

Probiotics · Metastasis · Free radical · Cancer · Carcinogen · Immune system

P. K. Prabhakar
Department of Medical Laboratory Sciences, Lovely Professional University, Phagwara, Punjab, India
e-mail: Pranav.16113@lpu.co.in

Y. Mishra
Department of Zoology, Shri Shakti Degree College, Ghatampur-Kanpur, UP, India

V. Mishra (✉)
School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India
e-mail: vijay.20352@lpu.co.in

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2.1 Introduction

Cancer, one of the most widespread diseases, is of almost 200 different types and affects more than 60 different human organs (Song et al. 2015). Cancer is the second leading cause of death in the different parts of the world with the total estimated number of 14.1 million new cases as well as 8.2 million deaths, which are expected to increase up to 21 million cases and 13.2 million deaths by 2030. The main reason for the cancer is the progressive accumulation of mutation in the genetic material. There are three different types of genes in which mutation leads to cancer and those genes are: protooncogenes, tumour suppressor genes and DNA repair system. Most important characteristics features of the malignant tumours are unregulated cell division, contact inhibition, loss of anchoring nature, irresponsive to growth factors and power to infect nearby cells and tissues (Otake et al. 2006; Balducci 2007; Luo et al. 2009; Hanahan and Weinberg 2011). According to the data published by the World Health Organization (WHO), around 70% of deaths occur due to cancer in the middle- and low-income countries. One-third of the cancer deaths occur due to increased body mass index, inadequate physical activity, less fruit and vegetable intake in the diet, high alcohol intake, rise in tobacco consumption etc. There are reports that in most of the cases cancers are linked to the external factors (90–95% cases), whereas in only 5–10% cases it is genetic (Anand et al. 2008). The cancer is called as multifactorial disease as there are a number of factor responsible for it (Tian et al. 2010).

Human gastrointestinal tract (GIT) harbours roughly around 10^{14} different species of microbiota, which includes bacteria, viruses and smaller eukaryotes. These microbiotas start colonizing in the GIT tracts just after few months (Blaser 2014). All these microbes collectively known as gut microbiota and their collective genome are known as gut microbiome. The research explains that the association between these gut microbiotas and their host, human, is symbiotic. The microbiota helps human in many ways like metabolic activities, digestion of indigestible metabolites, synthesis of vitamin and other biomolecules, development of immune system, not allowing foreign pathogenic bacteria to grow and settle there and absorption of food components (Blaser 2014; Goulet 2015).

One of the most prominent features of cancer cells are the unregulated proliferation, and they are resistant to the apoptosis. Currently, there is no single effective cancer therapy available for cancer due to multifactorial aetiology of cancer, and a number of physiological and metabolic abnormalities inside the cell lead to the progression of cancer (Jain et al. 2010). Different types of cancer can be targeted by specific target such as some signalling molecules, regulatory signals, apoptotic signals, immune regulatory components, transcription factors, proteins, enzymes etc. These target molecules can be used and evaluated for the designing of new effective drug, which can combat cancer efficiently.

The WHO has suggested to focus on some alternative therapeutic strategies for the management of infections and diseases (Saarela et al. 2002). The association of modifiable health has proved that 50% of all cancers might be involving dietary components, which indicates that nutritional components and nutraceuticals play a

major role in the cancer management. In recent time, a number of dietary components and natural health products pull the attention of many scientists for the designing and development of commercial natural therapeutics. One of such treatment and therapeutic strategies are probiotic and its formulations, containing non-pathogenic microorganisms (living), which does not give any harmful effect in host rather protect and give benefit to the host against a number of diseases like cancers (Daniluk 2012).

2.2 Probiotics: An Outline

The term 'Probiotics' is derived from the Greek words *Pro* and *bios,* which mean for the life. The first concept for the probiotics and its benefits has been explained by Mechnikoff in 1907. He noted that there are some bacteria that might have a beneficial effect on health through natural gut microbiota (Metchnikoff 2004). However, the term 'Probiotics' was first given by Ferdinand Vergin in 1954. Various definitions are available for Probiotics, but the most appropriate definition is—these are a group of microorganisms, which reside inside the gut and help the host in various functions like metabolism, immunity, protection, supplement of vitamins and metabolism of some toxic food components (Gibson and Roberfroid 1995; Hamasalim 2016). These are the readymade available preparations, which contain live bacterial colonies such as lactococci, lactobacilli, bifidobacteria etc. (Table 2.1).

As per the WHO's nutritional guidelines, the probiotics can be explained as a formulation of living microorganism taken in an adequate quantity, which results in health benefit to host (Nolfo et al. 2013). Today, probiotics becomes an important ingredient of many traditional formulations and foods and hence the Food and Drug

Table 2.1 List of microorganisms used as probiotics

Genus	Species
Lactobacillus	*L. rhamnosus*, *L. acidophilus*, *L. plantarum*, *L. casei*, *L. delbrueckii* subsp. *bulgaricus*, *L. brevis*, *L. johnsonii*, *L. fermentum*, *L. reuteri*, *L. paracasei*, *L. crispatus*, *L. gasseri*, *L. lactis*, *L. salivarius*
Bifidobacterium	*B. infantis*, *B. animalis* subsp. *lactis*, *B. bifidum*, *B. longum*, *B. breve*
Bacillus	*B. coagulans*, *B. subtilis*, *B. cereus*, *B. laterosporus*
Lactococcus	*L. lactis* subsp. *lactis*
Enterococcus	*E. durans*, *E. faecium*
Streptococcus	*S. thermophilus*
Pediococcus	*P. acidilactici*
Leuconostoc	*L. mesenteroides*
Escherichia	*E. coli* Nissle 1917
Propionibacterium	*P. jensenii*, *P. freudenreichii*
Peptostreptococcus	*P. productus*
Akkermansia	*A. muciniphila*
Saccharomyces	*S. boulardii*

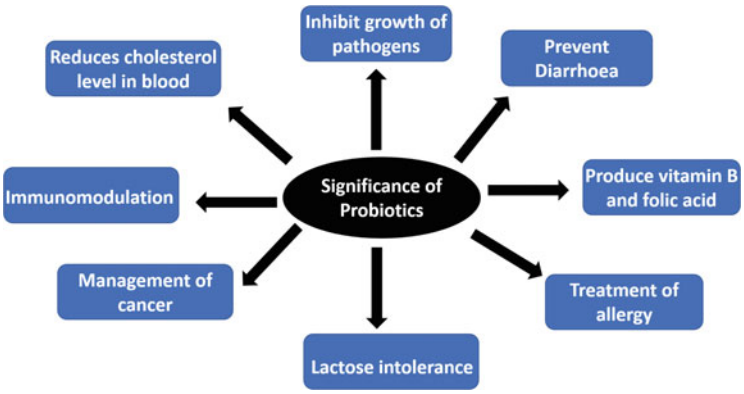

Fig. 2.1 Beneficial effects of probiotics on human health

Administration (FDA) endorses for their virtually null safety issues (Patel and Goyal 2013). Probiotics has already been used for the management and treatment of many diseases like inflammatory bowel syndrome, diarrhoea and other gastrointestinal diseases (Fig. 2.1). Probiotics are not only involved in the maintenance of intestinal epithelial homeostasis but also involved in the management of cancer via a number of ways (Daniluk 2012).

According to the International Scientific Association for Probiotics and Prebiotics (ISAPP), the probiotics are the living microorganisms, which when taken in the sufficient amount impart good effects on the health. Probiotics are differing from the live and active culture of microorganism. The commonest group of probiotic bacteria are from the genera Lactobacillus (LAB) and Bifidobacterium (BFB), a common indigenous gut microbiota of human GIT (Fijan 2014). Both groups of bacteria have the ability to survive in the intestine and also help host to regain the normal gut microbiota. These probiotic group of bacteria are ecologically diverse group of bacteria, which produce lactic acid as their primary metabolite after the carbohydrate metabolism (Masood et al. 2011). The mechanism of action and GIT disorder management properties of these two groups of probiotics are well understood, but there is lack of documented mechanism of the inhibition of progression of cancer by these bacteria. Probiotic *Lactobacillus* can be successfully used for the management of gastric diarrhoea in case of adult and child (Shida and Nomoto 2013). A group of scientists have reported that the *Lactobacillus* GG successfully managed milk allergy in kids through the increased secretion of IFN-gamma (Ozdemir 2010), whereas another group had shown the use of probiotics in the management of inflammatory bowel disease (Del Carmen et al. 2010). In this chapter, the use of probiotics as anticancer management strategies (Fig. 2.2) against different types of cancers has been discussed (Nolfo et al. 2013; Patel and Goyal 2013).

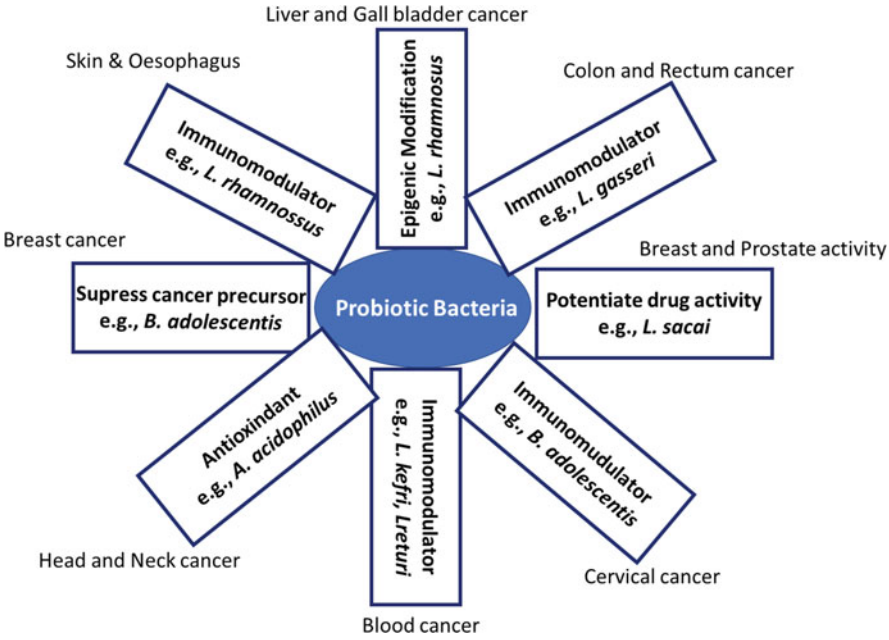

Fig. 2.2 Possible mechanism of action of probiotic bacteria against specific type of cancer

2.3 Mechanism of Action of Probiotics

Up to now, the relationship between probiotic with the health of human is very well established. The exact mechanism of action of probiotics is unknown, but it is expected to be multifactorial. A number of antagonistic activities of probiotics against pathogenic microorganisms and diseases have been postulated, which might be due to competition for food and nutrients for their growth and development, providing and improving gut barrier functions, competition for the shelter, surface to adhere, release of antimicrobial components and inducing immune system and its efficacy (Bermudez-Brito et al. 2012; Khalighi et al. 2016).

Among all these mechanisms, the most preferred way by which probiotics affect the growth of pathogenic microorganism is through inhibition of the growth of these microorganisms. For example, the probiotics colony may consume the available monosaccharides, and hence it will not be available for the pathogenic microbes, which are completely dependent on these monosaccharides for their growth and development such as *Clostridium difficile*. This results in the stopping of the growth of pathogenic microbe in the GIT tract and reduces the prevalence of such microbes in the gut (Wilson and Perini 1988). Another important mechanism by which probiotics work is through enhancing the gut barrier function by giving a competitive exclusion for their attachment to the mucosa released by the epithelial cells of gut. Maintaining the homeostasis of gut epithelial layer in the GIT is major defence

mechanism provided by probiotics. This is important as once this barrier function is breached, pathogenic bacteria and the antigen from food can reach up to submucosa and induce the inflammatory responses, which can result in gastric disorders; one of such disorder is inflammatory bowel disease (Hooper et al. 2003; Sartor 2006). Damage of the intestinal barrier leads to the pathogenic bacterial translocation, which is a primary inducer of the different types of cancers and other complications. A number of research group have documented that the probiotics like *Lactobacillus rhamnosus* strain GG and *Lactobacillus plantarum* 299 inhibited the attachment of enteropathogenic *Escherichia coli* in the GIT tract lumen (Wilson and Perini 1988; Pizzorno and Murray 2012). In addition, *Lactobacillus* bacteria also induce the expression of a number of tight junctions signalling proteins such as E-cadherin and Beta-catenin to reinforce the intestinal barrier integrity. Probiotics also keep the intact intestinal barrier integrity through the attachment and anchoring to the mucosal surface of gut. Several lactobacillus species displayed surface adhesins that help them in the integration and association with the glycoprotein, such as mucin, secreted by the intestinal epithelial cells and competitively exclude the pathogenic bacteria from the epithelial surfaces (Bermudez-Brito et al. 2012).

Another way of probiotic mechanism of action for the modification of gut microbiota is through the synthesis and release of high-molecular weight antimicrobial molecules such as bacteriocins and low-molecular weight organic acids such as lactic acid and acetic acids (Bermudez-Brito et al. 2012). These bioactive compounds have shown to exhibit the inhibitory activity against gram positive pathogenic microbes, such as *Helicobacter pylori*, which are involved in a number of gastric disorders. The main mechanism of action of these acids is by reducing the intracellular pH or the accumulation of ionized form of organic acids, which disrupt the pH homeostasis of the pathogen and ultimately affect the growth of the microbes (Russell and Diez-Gonzalez 1997; Ouwehand and Vesterlund 2004). Bacteriocins are proteinaceous compounds produced from the bacterial cells and have antibacterial activities. The probiotic bacteriocins are lactacin B (*Lactobacillus acidophilus*), plantaricin (*L. plantarum*), bifidocin B (*Bifidobacterium bifidum NCFB 1454*), nisin (*Lactococcus lacti*) etc. (Nielsen et al. 2010). These compounds are even effective against food-borne pathogens and work by a common mechanism of action by forming pore in the plasma membrane or inhibition of cell wall synthesis. Bifidocin B released from *Bifidobacterium bifidum NCFB 1454* has shown its efficacy against variety of pathogenic bacteria such as *Salmonella enterica* ser. Typhimurium SL1344 and *E. coli* C1845 (Nielsen et al. 2010).

2.4 Role of Probiotics in Cancer

It is a known fact that the progression of various categories of cancers can be decreased by modulating life styles such as stopping cigarette smoking and taking a balanced nutritional diet (Pool-Zobel 2005). Much more attention has been put forward nowadays for the reduction of cancer's risk factors via diet variation

especially the consumption of prebiotics (a category of fibrous food, which promotes the growth of favourable good bacteria) and probiotics.

Some of the recent reports have shown that there is an inverse relationship between the carcinogenesis risk and the administration of cultured milk products-like milk, yoghurt or fermented milk (Davoodi et al. 2013). There exist encouraging evidences, which suggest that the specific types of probiotics (*Lactobacillus*) have a significant role in the cancer management through the increased IL-2 and IL-12 cytokine production, increased expression of antioxidant enzymes such as superoxide dismutase and catalase, reduced glutathione and increased antiageing factors. They also lead to the decreasing DNA damage, reduction in inflammation, tumour size and procarcinogen protein and enzyme production as well as polyamine contents.

2.4.1 Mechanism of Cancer Management with Probiotics

Different experimental proofs showed the efficacy of probiotics, alone and in formulation, for the management of cancer in human and murine model (Rafter 2002; Jan et al. 2002; Baldwin et al. 2010). The work of Baldwin et al. (2010) has shown that the *Lactobacillus acidophilus* and *Lactobacillus casei* species induced the apoptosis in colorectal carcinoma cell line (LS 513), suggesting their anticancer activity. *Propionibacterium freudenreichii* also induces the death in the human colon and gastric cancer cell lines, mediated through the release of short-chain fatty acids (SCFA) into the culture media. Bacterial culture supernatant along with short-chain fatty acids showed an induction effect on apoptosis through the production of reactive oxygen species, loss of mitochondrial transmembrane potential, activation of proapoptotic caspase-3 and nuclear chromatin condensation (Jan et al. 2002). *Lactobacillus* spp. also induced the proapoptotic cytotoxic effect on leukaemia and colon cancer cell lines and anti-inflammatory effect on macrophages at the molecular level.

Shyu et al. reported that *Lactobacillus* spp. from dairy products secreted metabolites with cytotoxic and anti-inflammatory effects, and they strongly suggested that the increased cytotoxicity for HT-29 and HCT116 cells may be associated with an up-regulation of the early apoptosis gene markers *cfos* and *cjun* (Shyu et al. 2014). Some probiotic strains have been reported to influence haematological cancers such as *L. reuteri*, which enhanced TNF-induced apoptosis in human chronic myeloid leukaemia-derived cells (Iyer et al. 2008). Le et al. demonstrated that the symbiotic association between prebiotics and probiotics considerably assists the apoptotic response to a genotoxic carcinogen (Le Leu et al. 2005).

The exact mechanism of the use of probiotics in the preventing, treating and reducing the cancer progression is not very well established, and these topics need to be further elucidated. However, there are a number of scientific reports available, which establish some mechanistic role of probiotic in the cancer prevention and management. These mechanisms are performed through the (a) gut microbiota

modification, (b) improvement of function of gut barrier, (c) degradation of the carcinogenic compounds and the protective effect of intestinal epithelial DNA damage and (d) activation and enhancement of immune and inflammatory process in the body.

2.4.1.1 Gut Microbiota Modification

Probiotics have an important function on the gut microbiota by maintaining the balance and suppressing the growth of pathogenic and carcinogenic bacteria into the gut. A number of gram positive probiotic bacteria have the ability to produce antimicrobial proteins and peptides, acetic acid, lactic acid and propionic acid, which decreased the intestinal pH and reduced the growth and development of pathogenic gram negative bacteria (Suskovic et al. 2010). There are a number of other studies that support these facts and show that various *Lactobacilli* strains have shown antagonistic activities against the gram negative *H. pylori*, which is responsible to cause gastric cancer (Oh et al. 2002; Chen et al. 2012; Kuo et al. 2013). Adding to the current reports, another report shows that some of the strains of *Lactobacillus* release lactic acid, which have shown a significant inhibition of *Salmonella enterica* (Makras et al. 2006). In the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) model, an increase in *Lactobacilli* (LAB) and a reduction in the faecal coliforms and clostridia have been found due to *L. acidophilus* or *L. casei* (Chaikham et al. 2012). Li et al. reported that the gut microbiota has been shifted towards the beneficial bacteria such as *Prevotella* and *Oscillibacter* due to the probiotics (Li et al. 2016). These strains are known source of anti-inflammatory metabolites, which lead to the reduction of Th17 polarization and increases the anti-inflammatory Treg/Type 1 regulatory T (Tr1) cell differentiation in the gut.

2.4.1.2 Improvement of Function of Gut Barrier

As we know, our gut possesses a number of commensal bacteria that are mutually benefitted with the host. The maintenance of the intact gut epithelial lining is important for keeping a peaceful relationship between the commensal microorganisms and the hosts and also to protect from the pathogenic bacteria and pathobionts. Dysbiosis is an imbalance or alteration in the amount and the number of gut microbiota, which results in the pathological conditions and disease states. It damages the symbiotic physiological relationship between epithelial cells and the commensal bacteria and leads to offending the barriers such as inflammatory pathologies and also may induce the carcinogenesis and its progression (Roy and Trinchieri 2017). Commane and co-workers have shown that the fermentation product extracted from the probiotic and prebiotic bacteria stops the damage of the gut epithelial barrier, while Ko and co-workers have reported that *L. plantarum* reduced the transepithelial resistance of Caco-2 cells (Commane et al. 2005; Ko et al. 2007). Other reports showed that the administration of probiotics induced the expressions of mucin protein, MUC2 and MUC4, a tight junction protein. These mucin proteins enforced and enhanced the physiological role of intestinal epithelial barrier (Bermudez-Brito et al. 2012). These results suggested the significance of

probiotics in maintaining the integrity of mucous layer in the GIT, which is required for performing the intestinal barrier functions.

2.4.1.3 Degradation of the Carcinogenic Compounds and the Protective Effect of Intestinal Epithelial DNA Damage

There are a number of carcinogens available that can cause cancer via mutation or by affecting DNA sequences. These carcinogens are 2-dimethylhydrazine (DMH) and N-nitrosodimethylamine (NDMA). Probiotics along with normal gut microbiota have the ability to metabolize these carcinogens and have been studied in detail (Yu and Li 2016). Freeze–dried probiotic formulations of *L. rhamnosus* GG, *Bifidobacterium animalis* CSCC1941, *Streptococcus thermophilus* DD145 and *L. acidophilus Delvo* Pro LA-1 strains have shown an inhibition of tumour in DMH-induced intestinal tumour rat models when compared to the control group (Mcintosh et al. 1999). In case of colon epithelium, the probiotics have proven to show the decrease in the DNA damage caused due to site-directed DNA mutagenesis or adduct formation on colonic epithelium (Horie et al. 2003; Oberreuther-Moschner et al. 2004; Yeh et al. 2007; Kumar et al. 2010).

The 5-fluorouracil (5-FU) is a carcinogenic mutagen, which leads to mutation in the DNA. An experiment with rat intestinal epithelial cells has shown preventive result of probiotics against enterocyte apoptosis and loss of intestinal barrier function caused by 5-FU (Prisciandaro et al. 2012), while another in vivo study with rat has revealed that the combination of resistant starch and *B. lactis* induced the apoptotic response for the carcinogen-induced DNA damage of the rat colorectal cells (Le Leu et al. 2005).

The administration of probiotics or synbiotics significantly decreased the activities of intestinal procarcinogen enzymes, which was associated with colonic carcinogenesis in experimental animal models (Rowland et al. 1998; Nakanishi et al. 2003; De Moreno and Perdigon 2005). Administration of a probiotic bacterium, *Bacillus polyfermenticus*, significantly reduced the number of DMH-induced ACF in F344 rats, when compared to the controls (DMH-treated, no probiotics supplementation) (Park et al. 2007). Furthermore, a study conducted by Ohkawara et al. reported that the probiotics-treated group showed significantly less DMH-induced DNA damage, less blood lipid peroxidation and increased Total Radical Trapping Antioxidant Potential (TRAP) by 9.3% versus the controls (Ohkawara et al. 2005).

2.4.1.4 Activation and Enhancement of Immune and Inflammatory Process in the Body

A number of researches reveal that the probiotics enhance the activities of immune system in cancer patients. Lakritz et al. (2014) explained that the probiotic *Lactobacillus reuteri* ATCC-PTA-6475 strain inhibited cancer in mammary gland in wild-type and FVB strain erbB2 (HER2) mutant mice through CD4+ and CD25+ T-lymphocytes. Another group of report has also revealed that the supplementation of probiotic *L. casei* improves the activities of natural killer cells and T-lymphocytes and also enhances the phagocytic activity of macrophages, which results in the inhibition of cancer progression in mice animal model with different types of cancers

(Yamazaki et al. 2000; Takagi et al. 2001; Foo et al. 2011). Oral intake of *Bacillus polyfermenticus* in a patient with colon cancer has shown stimulated production of IgG and also modulated the proliferation and development of CD4þ, CD8þ or NK cells (Foo et al. 2011).

Some other studies also speculated that the food supplemented with probiotics has an impact of the cell signalling processes in cancer patients. *L. reuteri* has shown an inhibitory effect on carcinogenesis through the suppression of NF-κB-dependent regulatory genes such as the genes involved in cell division, e.g. Cox-2, cyclin D1 and genes involved in cell survival, e.g. Bcl-2, Bcl-xL (Lee et al. 2008). A separate study on 150 colorectal carcinoma patients taking probiotic formulations has showed a noticeable decrease in the complications of disease mediated by the reduction of p38 mitogen-activated protein kinase signalling pathway when compared with the control group of human (Liu et al. 2012). In addition, a novel purified *L. acidophilus* 20079 exopolysaccharide, LA-EPS-20079, inhibits in human colon cancer by regulating both apoptotic and nuclear factor-κB (NF-κB) inflammatory pathways (El-Deeb et al. 2018).

Furthermore, inflammation leads to the development of cancer mediated through the processes that involve genotoxicity, aberrant tissue repair, proliferative responses, invasion and metastasis. In most of the cases, the inflammation-associated carcinogenesis involves the transcription factors, signal transducer and activator of transcription 3 (STAT3) and NF-κB, modulating the release of inflammatory molecules like cytokines, interleukins and interferons (Elinav et al. 2013). For instance, Matsumoto et al. have also reported that *Lactobacillus* and VSL#3 probiotic supplementation have reduced and slowed the transition from inflammation to dysplasia in an experimental colitis-associated cancer rat model (Matsumoto et al. 2009).

2.5 Commonly Used Probiotics as Cancer Treatment

The two most commonly used microbes as probiotics are lactic acid bacteria and bifidobacteria. Some other bacteria and yeast also have beneficial effect on host. One of the main reasons to use microbes as probiotics is due to their immunomodulatory effect that was first postulated around 100 years ago by Metchnikoff (Anukam and Reid 2007). Nowadays scientific community has shown their interest in the use of probiotics in intestinal disorders especially colon carcinomas. Orlando and co-workers have reported that the administration of *Lactobacillus* GG decreased the polyamine synthesis in two HCG-27 and DLD-1 cancer cell lines (Orlando et al. 2009). Kim et al. found the anticancer activities of *B. adolescentis* SPM0212, which inhibited the proliferation of three colon cancer cell lines: HT-29, SW480 and Caco-2. The probiotic strain also suppresses the TNF-α production in a dose-dependent manner (Kim et al. 2008a, b). Consumption of *Lactobacillus* or *Bifidobacterium* containing milk products like yoghurt and fermented milk decreases the risk of colon carcinogenesis (Shahani and Ayebo 1980). *Lactobacillus* intake also reduces the urine and faeces associated carcinogenesis, which occurs due to

consumption of carcinogen containing cooked meat (Lidbeck et al. 1992a, b). It might be possible that the *Lactobacillus* increases the excretion of carcinogenic compounds through the attachment in the GIT. There are a number of ways by which probiotics help in the colon cancer treatment and its management. It is well listed that the probiotics interact with the host system in different places like GIT, skin and urinary tract and produce health benefits. The experimental data suggest that *L. acidophilus*, *L. salivarius*, *L. plantarum*, *L. rhamnosus*, *L. kefiri*, *L. casei*, *L. delbrueckii*, *B. infantis*, *B. breve*, *B. longum* and *S. thermophilus* help in the colon cancer management through various mechanisms (Drago 2019; Sharma 2019) including:

a. Degradation of carcinogenic chemicals,
b. Influencing epithelial repair and gastric barrier and increasing its function by enhancing the production of mucin, defensins and immunoglobulin A (IgA),
c. Decreasing pH of GIT,
d. Modulating the responses of proinflammatory cytokine and chemokine,
e. Inducing apoptosis in tumour cells, reducing dysbiosis and maintaining eubiosis,
f. Increased production of cytokines (IL-2 and IL-12), antioxidants and anti-angiogenic factors,
g. Induction of cytokines production promotes tissue repair,
h. Generation of metabolites like short-chain fatty acids (acetate, butyrate and propionate) having inductive effect on the epithelium and immune cells,
i. Selective exclusion of pathogenic and tumourigenic bacteria (bacteriocins),
j. Decreases biofilm formation through toll-like receptors, and synergistic effect with antitumour drugs for improving their effects.

There are a huge number of literatures, which show the effect of probiotics of different types of cancer. Here is the list of some of the recent studies on cancer where cancer has been managed or treated through the administration of probiotic bacteria (Table 2.2).

2.6 Conclusion

Probiotics are a group of microorganisms, which possess important functional attributes and fulfil most of the requirements of human body. The probiotic microorganism has gained medical significance due to its beneficial role on our health. Oral intake of probiotics microbes has shown multiple effects like the establishment of normal gut microbiota, development and improvement of gastric functional barrier, development of some of the immunological organs, production of vitamin and folic acid, inhibition of pathogenic microorganism's growth and anticarcinogenic effect. Probiotics not only improve systemic immunity or anti-inflammatory activities but also decrease the incidence of a number of chronic diseases like cancer, diabetes, allergy, cardiovascular disease, diarrhoea, gastric

Table 2.2 Probiotics used for the management of tumour and their mechanism of action

Probiotic strain	Type of cancer	Type of activity/response
L. acidophilus	Glioblastoma and breast cancer	Cytotoxic effects
	Ehrlich ascites carcinoma (EAC)	Reduced tumour volume
	Lung cancer	Reduction in tumour size and increased survival rates
	Colorectal cancer	Antiproliferative and anticancer
L. plantarum	Glioblastoma and breast cancer	Cytotoxic effects
	Murine adenocarcinoma	Antitumour response
	Colon cancer	Reduction of tumour incidence, suppression of COX-2 expression
	Sarcoma	Antiproliferative and immunomodulatory
L. plantarum LS/07	Breast cancer	Antiproliferative and immunomodulatory
L. rhamnosus	Murine adenocarcinoma	Antitumour response
	Colon cancer	Reduction of tumour incidence, suppression of COX-2 expression
	Colorectal cancer	Reduction of tumour incidence
	Cervical and colon cancer	Cytotoxic effects
	Cervical cancer	Antiproliferative effects
	Breast cancer	Cytotoxic effects
L. crispatus	Cervical and colon cancer	Cytotoxic effects
	Cervical cancer	Antiproliferative effects
	Breast cancer	Cytotoxic effects
L. casei	Colon cancer	Apoptosis via JNK signalling pathway
	Colon carcinogenesis	Antimutagenic
	Colorectal cancer	Antiproliferative and immunomodulation
L. casei BL23	Colorectal cancer	Modulation of regulatory T cells
L. lactis	Breast cancer	Inhibition of cancer growth
L. salivarius	Colorectal cancer	Decreased cancer incidence
L. fermentum	Colorectal cancer	Anticancer
L. johnsonii	Colon cancer	Anticancer
L. lactis subsp. lactis	Stomach cancer	G0/G1 cell cycle arrest and apoptosis
	Colon, cervical, gastric, and breast cancer	Apoptosis, antitumour effect
L. lactis	Lung, breast, and colon carcinoma	Decreased cell proliferation; Antiproliferative
B. lactis	Colorectal cancer	Antiproliferative
B. bifidum	Colorectal cancer	Antiproliferative
B. infantum	Colorectal cancer	Antiproliferative

disorder etc. The possible mechanism of action of probiotic against hypercholesteremia is through deconjugation of bile, binding of cholesterol with small intestine, cholesterol utilization and incorporation into probiotic microbe's cell membrane, and transformation of cholesterol into other metabolites. Similarly, the possible mechanism against cancer is through the modulation of gut microbiota, improvement of gut functional barrier, immune components, signalling system, reduction in inflammatory reactions etc. But still the uses of probiotic against diseases are still not very acceptable due to a number of factors. Since the use of animal model does not reciprocate human body so the effect may vary in human even after animal trial. Hence it is strongly recommended that more elaborated and long-term human complementary studies should be performed for better understanding of the efficacy of probiotics.

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Probiotics and Cancer: Boosting the Immune System

3

Prashant Upadhaya, Prachi Kharkar, Abhinandan Patil, Shivaji Pawar, John Disouza, and Vandana B. Patravale

Abstract

The concept of use of functional food/probiotics in the recent years as adjuvants for prevention and treatment of cancer has been on rise owing to their capabilities to restrain the host immune response and modulate the intestinal microflora. Numerous studies have proved that probiotics can be of potential use in the prevention and treatment of cancer through microbiota and immune modulation, condensed bacterial translocation, enhanced gut barrier function, anti-inflammatory, anti-pathogenic activity, reduced tumour formation, reduced metastasis, etc. Probiotics refer to live microbial incorporations available in a variety of food, mainly the fermented ones. Other than that, bacteria producing lactic acid, perceived to have useful properties such as resistance to pathogens, improving lactose digestion, etc. are also commonly referred as probiotics. The present chapter discusses the role of various probiotic strains in cancer and summarizes the important findings in relation to the probiotic mediated suppression of gastrointestinal and extra-intestinal cancers.

Keywords

Probiotics · Cancer · Immune system · Immunosurveillance · Lactobacilli

P. Upadhaya · P. Kharkar · V. B. Patravale (✉)
Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology, Mumbai, Maharashtra, India
e-mail: php15pg.upadhaya@pg.ictmumbai.edu.in; bpt12pb.kharkar@pg.ictmumbai.edu.in; vb.patravale@ictmumbai.edu.in

A. Patil · S. Pawar
Centre for Interdisciplinary Research, D. Y. Patil University, Kolhapur, Maharashtra, India

J. Disouza
Tatyasaheb Kore College of Pharmacy, Kolhapur, Maharashtra, India

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3.1 Cancer: Role of Immune System

The human immune system is capable of specific obliteration of tumours without any toxicity to the normal tissues. In addition, the immune system can also preserve this memory for a long term and prevent the cancer reoccurrence via a process known as 'immunosurveillance'. The research in the immuno-oncology field has presented us with concrete substantiation that immunosurveillance can not only identify tumours but also stop and prevent reoccurrence of the same for a long term (Finn 2012). The idea of immunosurveillance was accredited in the 1990s when research involving knockout mice animal models corroborated the reality of the concept of cancer immunosurveillance in spontaneous and chemically induced tumours. Since then the dominant roles of the effector cells of the immune system such as natural killer T cells, B cells, perforin and interferons (IFN) (I and II) were elucidated in cancer immunosurveillance (Dunn et al. 2002, 2005; Kim et al. 2007). The target specificity of the immune response exists in the differentiation ability of the antigens of the tumour. Other than these, the viral proteins in tumour instigated by viruses, the self-expressed proteins from the tumour surface, and the mutated and the non-mutated proteins from the onco genes exhibit virtuous targets for immunosurveillance (Finn 2012). The cancer immunoexpurgation resulting from immunosurveillance generally follows three essential phases, i.e., elimination, equilibrium and escape (Dunn et al. 2002). The cancer cells are initially eliminated by the effector cells such as natural killer cells and IFN-γ from the innate response of the immune system. Eliminating the transformed cells can result in decreased immunogenicity thereby rendering the tumour resistant to effector cells of the immune system in the equilibrium phase. Ultimately, during the progression of the tumour when diagnostic methods are able to detect the size of the tumour, factors such as tumour-derived soluble factors can instigate various mechanisms in the tumour microenvironment for the escape from the attack of the immune system (Kim et al. 2006).

In the recent years, research has proved the role of probiotics in exalting the immune response for the fight of cancer (De Leblanc et al. 2007). Many strains of probiotic organisms have the potential to impact innate mechanisms of defence such as phagocytosis (Schiffrin et al. 1995, 1997; Pelto et al. 1998; Arunachalam et al. 2000). Perdigon et al. in 1988 proved the potential of *L. acidophilus* and *L. casei* in systemic immunostimulation by the increase in the phagocytosis capability of murine peritoneal macrophages (Perdigon et al. 1988). Similarly, probiotic organisms also help in regulating the activity of natural killer cells (Gill et al. 2001), enterocytes and cytokines production (Lammers et al. 2002). Probiotics also impact the adaptive immunity by stimulating the production of IgA (Link-Amster et al. 1994; Fukushima et al. 1998; Isolauri et al. 2000; Park et al. 2002), dendritic cells (DCs) and Treg cells (Christensen et al. 2002; Braat et al. 2004). In this chapter, we shall discuss the potential and use of probiotics for improving the immune system in cancer condition.

3.2 Probiotics: Improving Immunity

Probiotics exert several beneficial effects on the host immune system. These effects include blocking pathogenic bacterial effects by producing bactericidal substances and competing with pathogens and toxins for adherence to the intestinal epithelium. Probiotics have been found to improve the innate as well as adaptive immunity by modulation of DCs, macrophages, and T and B lymphocytes functions via toll-like receptor-regulated signalling pathways. DCs being the antigen presenting cells play an important role in both adaptive and innate immunity. They also possess the properties of activating naïve T cells and have an important role in guiding the helper T cells towards the regulatory pattern or Th1 and Th2 (Lammers et al. 2002). The Th1 responses of the immune system decisively rely on the proficiency of the DCs to produce interleukin (IL)-12. The same are characterized by the production of IFN-γ and IL-2. The Th2 immune responses generally involve humoral immunity and IL-4, IL-5, IL-6 and IL-13 (Link-Amster et al. 1994; De Leblanc et al. 2007). Some of the prominently studied mechanisms of immunity involve the production of the IFN-α or induction of indoleamine 2,3-dioxygenase, an immunoregulatory enzyme with prime role in the interaction between the T cells and the DCs (Fukushima et al. 1998; Park et al. 2002).

In a recent study, it was demonstrated that probiotics activate the innate immunity and trigger the adaptive immune responses. It was found that a mixture of probiotics including strains of *L. reuteri*, *L. acidophilus*, *B. bifidum*, *L. casei* and *Streptococcus thermophilus* stimulated regulatory DCs expressing elevated levels of (Transforming growth factor beta) TGFβ, (cyclooxygenase-2) COX2, IL10 and IFN-α, thereby promoting the generation of forkhead family transcription factor ($CD4^+Foxp3^+$) regulatory T cells and increasing the suppressor activity. Additionally, the aforementioned probiotic mixture induced T and B cell hypo-responsiveness and down-regulated the T helper cells without induction of apoptosis. It was also revealed in the in vivo studies that the aforementioned mixture supressed the intestinal inflammation, which involved association of $CD4^+Foxp3^+$ Tregs, induced by 2,4,6-trinitrobenzenesulfonic acid (Yan and Polk 2011).

3.2.1 Need of Probiotics in Cancer Therapy

Cancer is a cellular disorder in which a defective apoptotic pathway triggers uncontrolled cell growth. Normally, the cells grow and re-divide via mitosis, having control on the G_1 phase. The loss of control on the G_o pathway results in the condition called malignancy (Hamada et al. 2001). The advancements in medical science resulted in the development of new synthetic drugs against cancer. However, they have numerous side effects. With the advancement in the field of nutraceutical foods and nano-pharmacology, the first of its kind novel functional food to heal cancer is no far from the reality (Cencic and Chingwaru 2010).

Probiotics provide vital nutrients that impart health benefits and thus act as functional foods for dealing with many gastrointestinal disorders, and even cancer.

These are the class of microorganisms residing in the gastrointestinal tract and assisting the digestive and enzymatic function of the host (Ciorba et al. 2015). Humans receive IgA from the milk of the mother along with the inoculation cultures of the *Lactobacillus* (Soto et al. 2014). The journey of Lactobacillus is affected by the dysbiosis phenomenon between the pathogenic strains and probiotics microbial flora. It has been found that the concentration of these microbes falls in between the therapeutic window and has the potential to act as prophylactic and curative agents against different diseases of the host (Sandes et al. 2017).

3.2.1.1 Antioxidant Nature of the Probiotics for Prophylaxis Against Gastrointestinal Diseases and Cancer

Recently, it has been proven that these living non-pathogenic microbiota, after administration in appropriate doses act as antioxidant mediators thereby preventing and curing many diseases. They show different pharmacological and physiological activities that play a vital role in the human immune system (Wang et al. 2017). Chemicals released by the *Lactobacillus* strains e.g. SOD (superoxide dismutase) act as antioxidant mediators. These microbes also display the potential to chelate various metal ions during the digestion process e.g. *Lactobacillus casei* is proved to chelate Fe^{2+} or Cu^{2+}, due to its antioxidant nature (Amaretti et al. 2013).

Many signalling mechanisms at the cellular/molecular level assisted by the probiotic microbes involve multistep complex pathways (Table 3.1); Kelch-like erythroid cell-derived protein with CNC homology [ECH]-associated protein 1 (Nrf2-Keap1) deals with the transcriptional responses via exogenous sensitization (Smith et al. 2016) while the nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) deals by excessive oxidative stress loading in host cells. The anabolic processes involved in the cell growth cycle and cell division are generally governed by (mitogen-activated protein kinase) MAPK. Whereas its subtypes, p38-MAPK and c-Jun N-terminal kinases (JNKs) are linked with the diverse stresses encountered by the cell affected by osmotic shock and irradiations. The following table provides the various signalling pathways associated with the probiotic microbes in different host cells (Mishra et al. 2015; Wang et al. 2017).

Probiotics show antioxidant activity by producing metabolites like folate and glutathione (GSH). The folate regulates the vital metabolism signalling pathways involved in DNA replication, methylation and maintenance of wear and tear of the host cells. Evidence-based studies have shown that *Bifidobacteria* induces the folate

Table 3.1 Signalling pathway regulated by the probiotics in relation to antioxidant activities

Probiotics	Host cells	Signalling pathways
Clostridium butyricum	Rats	Nrf2-Keap1
L. rhamnosus GG	Cell line Caco2	MAPK
Bacillus amyloliquefaciens	Cell line 'IPEC-1 cell line'	Nrf2-Keap1
Lactobacillus sp. *SC4*	Mice	Nrf2-Keap1
Lactobacillus sp. CM	Cell line YAMC	MAPK
Lactobacillus sp. FC255	Cell line mice	Nrf2-Keap1

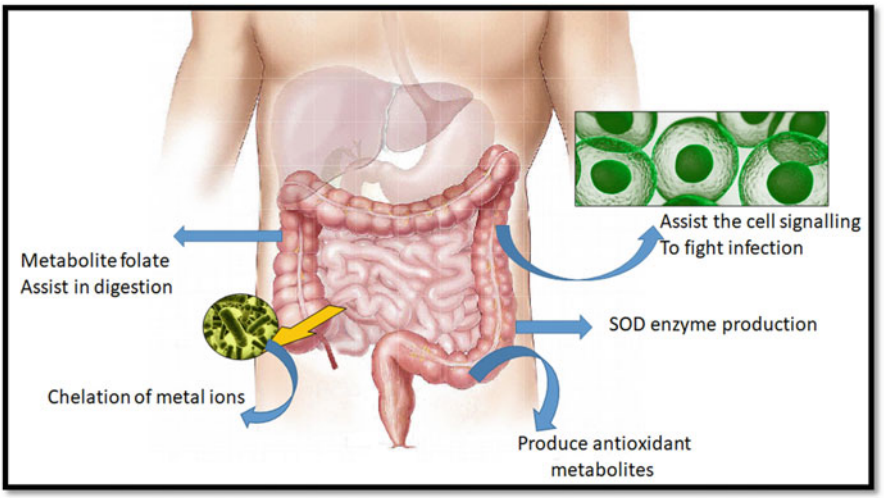

Fig. 3.1 Antioxidant activities exhibited by the probiotics involving various regulatory mechanisms

formation in animal models and human trials. Further investigations showed that the *L. fermentum* is capable of regulating the GSH system (Mikelsaar and Zilmer 2009; Wang et al. 2017). Thus, it can be concluded that probiotic treatments enhance the level of antioxidant metabolites.

Deficiency of vitamin B12 and folate promotes excessive oxidative stress in patients with type II diabetes. It has been shown that consumption of the yogurt, rich in *Lactobacillus* species, improves the level of the vitamin B12 along with the plasma folate relieving the oxidative damage in diabetes patients (Wang et al. 2017, Li et al. 2017a, p. 12). The detailed illustration of the antioxidant mechanism exhibited by the probiotics is given in Fig. 3.1.

3.2.1.2 Anticancer Nature of the Probiotics

Lactic acid bacteria also known as *Lactobacillus* are a genus from the probiotics group and are found to be useful in healing various common disorders and even cancer. The cancer healing abilities exhibited by the probiotics include various mechanisms, the most prominent being the suppression of cancer-causing mediators (Nami et al. 2014). This is regulated by preventing the carcinogenic metabolites from causing DNA alterations. Additionally, probiotics participate in the proper execution of the cell apoptosis, thereby preventing the cancer invasion by metastasis and growth of cancer stem cells (Motevaseli et al. 2017). The various mechanisms by which the probiotics act against cancer cells are illustrated in Fig. 3.2 and are discussed in detail in the following sections (Zhang et al. 2012; Yu and Li 2016; Motevaseli et al. 2017)

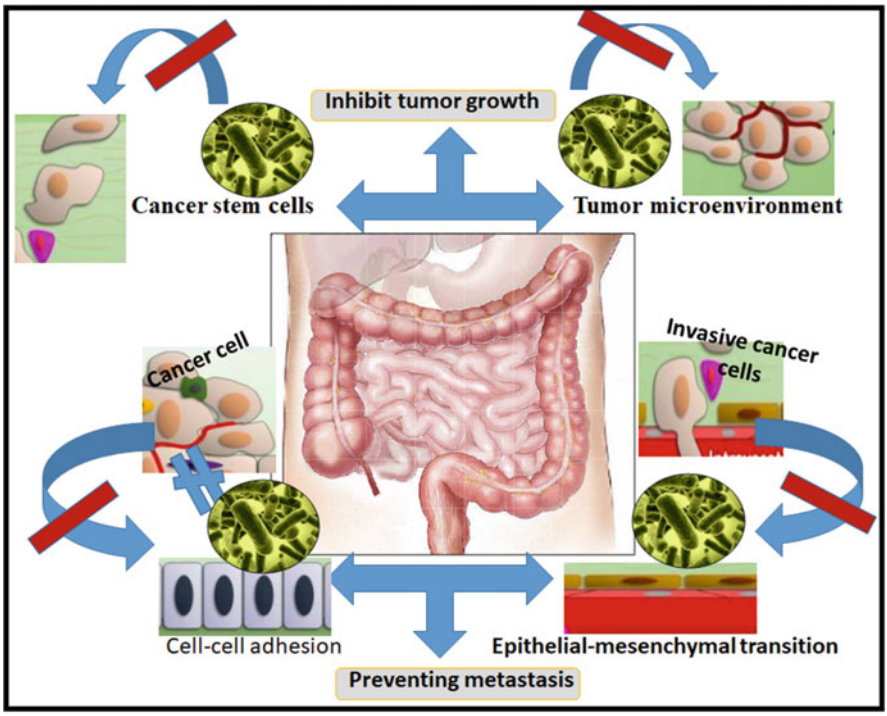

Fig. 3.2 Probiotic mechanisms inhibiting the infestation of the cancer

Protecting Host by Cell–Cell Adhesion

Normal physiology of the host tissue consists of tight junctions of the endothelial system showing cellular integrity. However, during the metastasis of cancer, these structures are weakened due to the damages in the scaffold proteins consisting of occludin and zonaoccludens-1 (ZO-1) (Motevaseli et al. 2017). Sometimes, the host cell membrane degrading agents such as matrix metalloproteinases assist the cancer cells to damage the structural integrity and invade other cellular structures and that is where probiotics play a role (Wan et al. 2014). The use of probiotics in a patient before surgery was found to enhance the liver barrier, thereby preventing the metastases (Eizaguirre et al. 2002; Dimitrov et al. 2014; Wang et al. 2017). *Lactobacillus* was found to be effective against cancer due to its cell adhesion mechanisms whereas *L. rhamnosus* decreased the overexpression of matrix metalloproteinase-2 in Caco2 cell line, thus maintaining cell–cell adhesion (Jan et al. 2002; von Ossowski et al. 2010; Motevaseli et al. 2017). Table 3.2 list cell–cell adhesion mechanism exhibited by probiotics.

Protecting Host by Inhibiting the Epithelial-Mesenchymal Transition (EMT)

The host's biological mechanism enables the epithelial cells to get polarized and develop affinity with the cell membrane. This results in the alteration of biochemical

Table 3.2 Cell–cell adhesion mechanism exhibited by the probiotics

Sr. no	Probiotics	Cell line/ host cell	Cell–cell adhesion mechanism
1	*L. acidophilus*	Human monocytes	Up-regulation of tissue inhibitors of metalloproteinases (TIMP)-1 as a tissue inhibitor
2	*L. rhamnosus* GG	Human monocytes	Up-regulation of metalloproteinases (TIMP)-9 as a tissue inhibitor
3	*L. rhamnosus* GG	MDA-MB-231 cells	Down-regulation of glucose transporter type 1 (*GLUT1)*
4	*L. acidophilus*	HT 29 cell line	Increase in intercellular adhesion molecule 5 (*ICAM5) expression*
5	*Lactobacillus species* (NCK2025)	HT 29 cell line	Up-regulation of TIMP-2
6	Kefir	4T1 cell line	Down-regulation of plasminogen activator urokinase
7	Kefir	Caco 2 cell line	Up-regulation of BCL2 associated X, apoptosis regulator (Bax)
8	Kefir	HT 29 cell line	Expression of tumour protein p53 (p53) independent of cyclin-dependent kinase inhibitor (p21) induction
9	*L. plantarum*	Caco 2 cell line	Translocation of zonula occludens-1 (ZO-1) in cell junction
10	*L. rhamnosus* GG	Caco 2 cell line	Up-regulation of Claudin-1 in cell junction

Table 3.3 EMT inhibition mechanism of the probiotics on the host cells

Sr. no	Probiotics	Cell line/host cell	EMT inhibition
1	*L. acidophilus*	CT 26 cell line	The up-regulation of the apoptosis TNF-factor based on ligand TNF-related apoptosis-inducing ligand (TRAIL)
2	*L. casei*	HT 29 cell line	Inhibition of TRAIL regulated metastasis
3	*L. casei*	CT 26 and HT29 cell line	Inhibiting the proinflammatory cytokines overexpression
4	*L. casei*	HT29 cell line	Inhibiting the microRNA 221 (miR-221) expression

processes affecting the migratory dimensions of the host cell against the invasion of cancerous cell. CXCR̆4 gene is found to be effective in enhancing the signalling pathway of EMT resulting in metastasis (Chen et al. 2012). The CXCR̆4 antibodies inhibit the adhesion to the cancerous cells preventing their migration. *L. acidophilus* is found to down-regulate the expression of CXCR4 and inhibit colon cancer in mice models (Chen et al. 2012; Motevaseli et al. 2017, 2018). Table 3.3 lists EMT inhibition mechanism of the probiotics on the host cell.

Inhibition of Tumour Microenvironment

The tumour microenvironment is developed by the communications that exist between the normal cells and tumour cells. The tumour-inducing cells in the microenvironment possess the ability to undergo various stages of tumour formation called tumourigenesis. These cells are non-malignant in nature and are related to the cells of the immune system and lymph nodal areas. A recent investigation carried out by the researchers demonstrated the anti-metastatic activities of *Lactobacillus* via alterations in the tumour microenvironment (Liu et al. 2015). Another study involving use of *L. casei* YIT018 in guinea pigs demonstrated their ability in suppressing the lymph node metastasis (Church and Galon 2015; Motevaseli et al. 2017). Table 3.4 lists mechanisms of tumour microenvironment inhibition by the proteins.

Inhibition of the Cancer Stem Cells

In the recent studies, it has been demonstrated that the 'cancer stem cells (CSC)' play an important role in haematological malignancies. Similar to normal stem cells, these cells possess abilities like self-renewal and prolonged survival and contribute to

Table 3.4 Mechanisms of tumour microenvironment inhibition by the probiotics (Orlando et al. 2009; Church and Galon 2015; Motevaseli et al. 2017)

Sr. no	Probiotics	Cell lines/ host cells	Tumour microenvironment inhibition pathways
1	*L. casei*	C47BL/6 mice	Suppresses tumour growth, protects against pulmonary metastasis
2	*L. casei*	C47BL/6 mice	Activation of natural killer cells as a cytolytic agent
3	*Lactobacilli species*	C47BL/6 mice	Suppression of metastasis
4	*Lactobacilli species YIT/9018*	Mice	Increase in IL-2 and IFN-γ level
5	*L. brevis*	BALB/c mice	Decreases the liver metastasis originated from the metastatic breast carcinoma
6	*L. brevis*	BALB/c mice	Increases the activity of the IFN-γ and IL-17 by activation of natural killer cells
7	*L. casei 431 CRL*	Wister rat	Antitumour activity linked to CD4+ and CD8+ lymphocytes
8	*L. casei* Shirota	HT29 cell line	Activation of natural killer cells
9	*L. rhamnosus* GG	HT29 cell line	Preventing the formation of free radicals with an enhancement of neutrophilic phagocytic activity
10	*L. casei* Shirota	Caco2cell line	Down-regulation of the angiogenic IL-1β factor
11	Kefir	Caco2cell line	Inhibiting the proangiogenic factor IL-6
12	*L. plantarum JDARSH*	HT 116 cell line	Preventing the formation of free radicals

Table 3.5 Cancer stem cells inhibition by the probiotics (Saxami et al. 2016; Motevaseli et al. 2017)

Sr. no	Probiotics	Cell line/ host cell	EMT inhibition pathways
1	*L. rhamnosus*	C47BL/6 mice	Inhibiting the (hypoxia-induced factor) HIF-1α signalling preventing metastasis
2	*Bifidobacterium breve*	C47BL/6 mice	Suppression of tumour cells by inhibition of inflammatory cytokine mediators
3	*L. plantarum*	HT 29 cell line	Activation of the natural killer cells by inhibition of inflammatory cytokine mediators
4	*L. crispatus*	HT 29 cell line	Down-regulation of HIF-1α signalling along with overexpression of *SFRP2*
5	*L. casei*	HT 29 cell line	Activation of the natural killer cells by inhibition of inflammatory cytokine mediators
6	*L. bulgaricus*	HT 29 cell line	Activation of the natural killer cells by inhibition of inflammatory cytokine mediators
7	*Bifidobacterium infants*	C47BL/6 mice	Suppression of tumour cells by inhibition of inflammatory cytokine mediators

cancer metastasis (Bui et al. 2015). These cells demonstrate heterogeneity in its functioning and show metastatic specificity to every organ system. The initiation and working of the CSC are due to the integral signalling pathways demonstrated by these cells against the host immune system (Llewellyn and Foey 2017). Alike stem cells, the self-transcriptional systems generated by CSC result in the induction of the hypoxic conditions in normal tissues. Further, this leads to regulations in signalling pathways involving multi-potent transcription factor Oct4, thereby initiating the migration of metastatic cells from one organ to the other. *L. rhamnosus* were observed to down-regulate the expression of hypoxia inducible factor-1α in breast cancer cell lines and colonic cancer cell lines (Bui et al. 2015; Motevaseli et al. 2017). Table 3.5 lists the pathways of cancer stem cell inhibition by the probiotics.

3.3 Probiotics and Cancer

The role of probiotics in cancer is recommended by several scientific indications and hypothesis that include upsurge in immune cell activation and suppression of the organisms converting procarcinogens. Below are some of the types of cancer and the study of probiotics associated with them.

3.3.1 Colon Cancer

Colorectal cancer is one of the leading cancers and nearly 862,000 deaths were reported in 2018 (Jan et al. 2002). The main reason behind the death owing to colorectal cancer is the leading dietary and behavioural risks taken by the patients which include obesity, low intake of fruits and vegetables, sluggish physical activity,

alcohol and tobacco consumption, etc. (Holscher 2017). Along with the diagnosis, the prognosis of the cancer is a critical issue. To cope up with these problems, few synthetic medicines have been discovered after several years of clinical trials. However, many have been discarded in the last phase of trials owing to host–drug incompatibility issues. Systemic administration is preferred rather than local for the treatment of colorectal cancer. This results in systemic toxicity and adverse effects on vital organs. Hence, there is the need for a drug that can be delivered by per-oral route, having local action, minimizing the systemic toxicity, controlling morbidity and preventing the mortality (Ohkawara et al. 2007).

Many of the functional food and nutraceutical formulations are available commercially but are limited in curing minor diseases or disorders (Cencic and Chingwaru 2010; Patil et al. 2015, 2019a). In order to tackle this problem there is a need for novel nutraceutical formulations derived from natural origin (Patil et al. 2018, 2019b). These formulations should be able to control the manifestation of various types of cancer and the activity shall be consistent. Probiotic formulations are widely available in the market for the treatment of bowel disorders. *Lactobacillus* are generally derived and isolated from milk of different milking animals (Patil et al. 2019c). Furthermore, cultures of the *Lactobacillus* are also available as powder formulations possessing biological activities like mucoadhesion (Ouwehand et al. 2003) along with properties of hydrophobicity and autoaggregation. They also maintain proper bowel movements so as to prevent gastrointestinal tract disorders. Many reported functional foods have the ability to trigger and initiate the innate immune system of the host and biological properties, including antimicrobial properties, which combat the activities of pathogenic microorganisms (Campbell et al. 2000). The abilities possessed by some probiotic strains to scavenge the free radicals enable them as anticancer mediators. The prepared formulations are ideal if they mimic the natural antioxidant mediators having long shelf life. However, short shelf life of probiotics makes them less demanding in the existing market. Thus, the *Lactobacillus* formulations with longer shelf life along with the surplus biological activities like antimicrobial, antioxidant and anticancer activities can be beneficial. These microbes with antioxidant activity help in triggering the innate immunity system along with the release of certain chemicals (proteins) eventually killing the cancer cells. The preliminary investigation regarding the effect of probiotics is evaluated in cell line studies. The antiproliferative, antioxidant and anticancer nature of the probiotics have been demonstrated successfully on colonic cancer cell lines. The following Table 3.6 shows the anticancer model developed by using the different cell lines (Choi et al. 2006; Russo et al. 2007; Kim et al. 2008; Orlando et al. 2009, 2012; Motevaseli et al. 2013, 2017; Nami et al. 2014; Dimitrov et al. 2014; Ghoneum and Felo 2015; Yu and Li 2016; Saxami et al. 2016; Malik et al. 2018; Patil et al. 2020).

Table 3.6 In vitro cell line studies using different probiotics

Sr.no	Probiotic formulations	Colon cancer cell line
1	*L. reuteri*	HCT-116, DLD-1
2	*L. kefiri*	HT-29
3	*L. casei*	CT-26, HT-29, WiDr, DLD-1 and CX-1 cells
4	*B. adolescentis*	DN
5	*L. acidophilus*	HT-29, WiDr, DLD-1 and CX-1 cells
6	*L. bulgaricus*	DN
7	*L. fermentum*	CRL-1831, Caco-2
8	*L. salivarius*	DN
9	*B. adolescentis*	DN
10	*L. rhamnosus*	HGC-27, Caco-2 and HT-29
11	*L. plantarum A7*	Caco-2 and HT-29

Where DN indicates—Data not available

3.3.2 Cervical Cancer

Cervical cancer is another malignant form of cancer observed in women globally. Cervical cancer is ranked fourth in the major causes of cancerous death among women worldwide (Lv and Wang 2018). No signs or symptomatic conditions are observed among the patients with cervical cancer at early stages; however, it has been proven to be fatal at the later stages eventually leading to death (Musa et al. 2017). In general, the microecological environment of the vagina is very sensitive where minute chronic changes may precipitate cervical diseases. The current research has shown that certain microorganisms may alter the microecological environment of female genital tract resulting in the development of cervical cancer (Yang et al. 2018a). Infection by the human papillomavirus (HR-HPV) causes diseases like colitis, high-grade cervical intraepithelial neoplasia along with cervical diseases in women (Chase et al. 2015).

The vaginal microflora observed in the case of patients with cervical cancer showed different counts of pathogenic strains such as *Staphylococcus epidermidis*, *Escherichia coli*, *Mycoplasma genitalium* and Enterococci species as compared to the control (Yue et al. 2015; Grmek Košnik et al. 2016). However, the exact correlation between the different microflora in normal female genital tract and that of cervical cancer is not yet elucidated (Sierra et al. 2018).

Probiotics especially *Lactobacillus* have an important role in cervical disorders. The major potential mechanisms exhibited by the probiotics against cervical cancer include:

(a) The autoaggregation mechanism that prevents adhesion of pathogenic strains to vaginal epithelial cells (VECs) inhibiting the conditions such as hyperplasia and wear–tear, thus decreasing the chances of any diseases or disorders.

Table 3.7 In vitro cell line studies using different probiotics

Sr. no	Probiotic formulations	Cervical cancer cell line
1	*L. crispatus*	CaSki cells
2	*L. casei*	DN
3	*L. gasseri* strains	Women, cervical cancer cell and Caco 2 cell lines
4	*Lactobacillus rhamnosus* HN001 (L1)	Th17 cells and HeLa cell lines
5	*L. bulgaricus*	DN
6	*L. crispatus*	HeLa cells
7	*L. casei*	CaSki and HeLa cell lines
8	*B. adolescentis*	DN
9	*L. plantarum*	Women; HeLa cell lines and HUVEC normal cells
10	*L. gasseri* and *L. crispatus*	Human normal fibroblast-like cervical (normal cervical) and HeLa (cervical tumour) cells
11	*Lactococcus lactis* and *L. casei*	HeLa and U14 cell lines

Where DN indicates—Data not available

(b) The ability to produce organic acids, which maintain the acidic microenvironment of the vagina inhibiting the invasion and growth of pathogens (Medina-Colorado et al. 2017).
(c) Release of chemicals such as bacteriocin, hydrogen peroxide and surface-active components, which play a vital role in inhibiting tumourigenic substances due to pathogens (Zadravec et al. 2015; Homburg et al. 2017).
(d) An effective immune sensitizer, assisting the proliferation of B cells in the bone marrow (Yao et al. 2007; Lee et al. 2010).

Furthermore, *Lactobacillus* has been found effective in hampering the metabolic pathway of the cancerous cells by the release of nitric oxide (NO) (Sandes et al. 2017). Also they act as an effective humoral immunity modulator, which mediates the proliferation of T cells in the thymus gland of the infants (Sandes et al. 2017).

The preliminary investigation regarding the effect of probiotics is evaluated by cell line studies. The antiproliferative, antioxidant and anticancer nature of the probiotics has been demonstrated successfully on cervical cancer cell lines is presented in Table 3.7 (Ribelles et al. 2013; Nami et al. 2014; Kim et al. 2015; Motevaseli et al. 2016; Seo et al. 2016; Jang et al. 2017; Li et al. 2017b; Wang et al. 2018; Yang et al. 2018b).

3.4 Breast Cancer

The past few years have witnessed a reduction in mortality rate due to breast cancer. However, breast cancer still remains one of the common cancers prevalent among the female population of the world. The fraternity of science working in the area of

breast cancer prophylaxis and therapy has thus started taking interest in the therapies other than chemotherapy for breast cancer. In vitro studies have demonstrated that breast cancer proliferation was inhibited by the use of isolated probiotics strains or supernatant from the cultures. It was also studied that heat killed cells, their cytoplasmic fractions and the live cultures namely *Enterococcus faecalis* and *Staphylococcus hominis* isolated from the breast milk caused cytotoxicity by means of apoptosis induction and arrest of the cell in the G0/G1 phase. It was also reported that the probiotics inhibiting the growth of the breast cancer also exhibited anti-inflammatory properties, suggesting their oncolytic property via immunomodulation (Han et al. 2015; Lee et al. 2015b; Nami et al. 2015; Hassan et al. 2016). The daily consumption of the strain *Lactobacillus acidophilus was reported to* increase the production of immunomodulatory cytokine IL12 in splenocyte culture. The same was stimulated by the tumour antigens in breast tumour bearing BALB/c mice (Yazdi et al. 2010).

3.5 Liver Cancer

The microbiome in the gut has been allied with the progression of liver disorders namely liver fibrosis (De Minicis et al. 2014), fatty liver disease (Raman et al. 2013; Wong et al. 2013) and in the recent years, liver cancer (Yoshimoto et al. 2013). In a recent study it was found that probiotics could inhibit the progression of the hepatocellular carcinoma in mice (Li et al. 2016). It was seen that upon feeding probiotics mixture to the mice with liver tumours, there was a shift in the gut microbiota, leading to the reduction of the tumour size in the liver of the mice. In addition to the above, it was also evident that there was a down-regulation in the angiogenic factors. Also, the level of the Th17 cells in gut and employment of Th17 to the tumour site were seen to be on the lower side in the mice treated with probiotics. The anticancer effect of the given probiotics was believed to have associated with the SCFAs-related pathway.

3.6 Other Cancers

Apart from the above-mentioned cancers, other types of cancers such as leukaemia, melanoma, lung cancer were also shown to have inhibited by the treatment of probiotics both in vivo and in vitro (Gui et al. 2015; Han et al. 2015; Lee et al. 2015a). Tuo et al. in their study demonstrated the antiproliferative activity of eight different strains of lactobacillus strains on leukaemia cells (Tuo et al. 2015). Similarly, Sivan et al. studied gut microbiota and immunotherapy on melanoma by the use of bifidobacterium species. It was found that the administration of the same caused reduction in the tumour volume of melanoma bearing mice (Sivan et al. 2015).

3.7 The Fate of Probiotics in the Animal and Clinical Studies

The anticancer effects of probiotics were determined by using various animal models such as rats and mice, the positive results of which lead to clinical trials on human volunteers (Fiala 1977; Chester et al. 1986; Foo et al. 2011; Asha and Gayathri 2012; Byelinska et al. 2015; El-Khadragy et al. 2018). Many studies were recently carried out using *L. acidophilus*, *L. amylovorus*, *L. brevis*, *L. bulgaricus*, *L. casei immunitas*, *L. casei*, *L. crispatus*, *L. delbrueckii*, *L. fermentum*, *L. gallinarum*, *L. helveticus*, *L. johnsonii*, *L. johnsonii LC-1*, *L. lactis*, *L. plantarum*, *L. reuteri*, *L. rhamnosus*, *L. salivarius*, *L. sporogenes* for analysing their anticancer activity using different animal models (Byelinska et al. 2015).

Mostly, probiotics have been employed as complementary synergistic mediators to manage intestinal disorders during chemotherapy and radiotherapy of cancer patients (Liu et al. 2011). *L. casei DN-114001* was investigated for its progression in the host body by the stool consistency studies and bowel movements physiology in patients undergoing radiation (Merenstein et al. 2010). Many researchers reported that the combination of *L. acidophilus* and *B. bifidum* minimize radiation-induced diarrhoea (Hickson 2011). It has also been reported that chemotherapy changes the human gut microbiota inviting the pathogenic strains such as *Clostridium difficile* in the gut of the patients suffering from colorectal cancer (Nakanishi et al. 2003; Shinnoh et al. 2013). The enteral administration of *bifidobacterium* and *Lactobacillus* not only improved the patient's intestinal environment but also minimized the side effects of radiotherapy (Walrand et al. 2012). Many studies showed that the administration of the probiotic formulations reduced post-operative trauma and intestinal infections (Liu et al. 2011; Zhang et al. 2012). Administration of probiotics also decreased the chances of tumour formations induced by aflatoxins, which are used as a marker for liver cancer (Kumar et al. 2012; Huang et al. 2017). The current research showed that the inclusion of probiotics as a nutraceutical agent reduces the risk of breast cancer in women after menopause. The consumption of these probiotics did not affect the level of the hormone in these women during their reproductive phase of the life (Bonorden et al. 2004). Probiotics were also found to be effective against the atypia form of colorectal tumours in patients consuming the *L. casei* for a period of 4 years (Gianotti 2010; Zhang et al. 2012).

3.8 Future Perspective and Conclusion

Cancer development is a progressive and protracted process involving complicated factors leading to metastasis. Epidemiological studies provide basis that probiotics have the potential to improve the lifestyle of the cancer patients and alter carcinogenesis. Research also shows that a number of probiotics have the potential of preventing cancer. These probiotics along with multiple health benefits possess anti-mutagenic and anti-carcinogenic properties. Such convincing research provides a strong basis for the acceptance of probiotics as chemo-supportive and chemo-preventive agents. However, the combination of the conventional treatment

approaches and probiotics have been known to play a critical role in the variance of the clinical results in the trials. Owing to the promising results, the future of probiotics seems bright and probiotic therapy is now being preferred in cancer. The effectiveness however is dependent on the species and strain of probiotics exerting its actions via multiple pathways in cancer treatment/prevention. This opens several possibilities in the area of genetically modified/engineered probiotic strains. Research is being conducted on engineered strains of organisms not only for therapy but detection too and the results seem convincing. Undoubtedly, the research investigations in the field of probiotics for cancer is in its infancy; however, the same has reassuring future in preventive therapy.

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Probiotics as Next Generation Strategy for Cancer Therapy

4

Anuradha Mishra, Pragyandip P. Dash, Afreen Usmani, Satya Prakash Singh, and Anup K. Sirbaiya

Abstract

Probiotics are the substances used for improving health status by improving the fitness of the intestine and overall host health. Research investigations confirm that correlation exists between intestinal microbiota and carcinogenesis. It has been found that normal homeostasis could be maintained by the consumption of probiotics. Further, probiotic administration can maintain sustainable physico-chemical conditions by reducing the number of harmful bacteria and in turn decreasing the level of enzymes like glucuronidase and nitroreductase. Recent research confirms the probiotic's significant role in the reduction of carcinogenic or mutagenic effects of consumed foods. Therefore, it indirectly improves or reduces the risk of cancer and other infectious disorders by boosting host immunity. It has been concluded that probiotics are not only useful in cancer prevention and inhibition of its progression but also possess therapeutic potential while screened against cancer cell lines. So, this chapter will focus its discussion on applications of probiotics as future drug therapy against broad array of cancers like colon, stomach, breast, cervix, and myeloid leukemia cells. Prospective use of this novel therapy could be next generation approach to offensive treatment techniques like chemotherapy or radiotherapy

A. Mishra (✉) · A. Usmani · A. K. Sirbaiya
Faculty of Pharmacy, Integral University, Lucknow, UP, India
e-mail: anuradha@iul.ac.in; afreenu@iul.ac.in; anupsir@iul.ac.in

P. P. Dash
Amity Institute of Pharmacy, Lucknow Campus, Amity University, Lucknow, UP, India
e-mail: ppdash@lko.amity.edu

S. P. Singh
City College of Pharmacy, Barbanki, UP, India

P. K. Deol (ed.), *Probiotic Research in Therapeutics*,
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Probiotics · Microbiota · Generation · Carcinogenesis · Chemotherapy

4.1 Introduction: Probiotics General Concepts

Abnormal and uncontrolled growth and spread of cells cause a group of diseases called cancer. If the spread is not controlled, it causes death. Cancer is the second most communal reason for loss in the USA following by heart disease (American Cancer Society 2019). The word probiotic derived from the Latin term *pro* ("in support") and the Greek adjective *bios* (biotic) ("life") meaning "in support of life" (Ezema 2013). Initially, Lilly and Stillwell (1965) developed a method to elucidate materials secreted by microorganism and now it is applied to animal feed supplements as microbes impacting a valuable effect to the growth of flora and fauna (Sperti 1971). Probiotics may be defined as "live organisms, which produces health benefit when consumed in ample quantity" (Hill et al. 2014). The outcome of probiotic treatment in prevention and reduction in cancer proliferation has been recognized long ago. In vitro findings on liver, breast, colorectal, cervical, and bladder cancer cell lines have confirmed that probiotics possess apoptotic, antiproliferative effects (Rossi et al. 2018; Russo et al. 2007; Nami et al. 2015).

Recent evidences suggested that both developed and developing countries are using herbal medicines in cancer therapy due to their nature of origin and limited side effects (Moteriya and Chanda 2017) but the main concern with these herbal products is their poor bioavailability (Watkins 2015). With the development of diverse in silico technologies, the preface of plant-derived bioactive agents into cancer therapy has altered the natural history of many types of individual cancer. Despite numerous advances in the field of cancer research, the world still continues to be in the clench of this horrible disease and there is a critical requirement to design well-tolerated anticancer therapeutic agents (Dutt et al. 2014).

Probiotics inclusive regimen is mostly used as a supportive drug throughout cancer therapy. Even though it is primarily effective as a preventive therapy, its role in the treatment of cancer is not properly implicit and requires further investigation. The mode of action of probiotics in the management of cancer includes improvement of gut barrier functions by the destruction of impending carcinogens, protection of intestinal DNA against degeneration, and augmentation of host immunity against inflammation (Nazir et al. 2018).

According to Ilya Metchnikoff, a nineteenth century scientist, within our gastrointestinal tract, there is a huge amount of bacteria known as the microbiome. In a healthy person, there are about a hundred trillion bacterial cells. The ratio of microbes in the gut to human cells is three to one while some are harmful; these are continuously checked by good bacteria known as "commensals." These bacteria improve digestion and therefore maintain good health, and strengthen immune systems. So, if we enhance the mix up of microorganisms in the gut, ultimately we can increase response to disease. Later, he claimed that aging is caused by bad

microorganisms in the gut and suggested people to eat more probiotics/yogurt. Many other researches also claimed that the bacteria in yogurt/probiotics are good for cancer patients (Mackowiak 2013). According to De Vita cancer textbook "The gut microbiota regulates the responsiveness to anti- PD-1 cancer therapy." PD-1 are the new immune checkpoint inhibitors. Rosenberg and colleagues reported that cancer patients with healthier microbiota were able to produce strong antitumor immune responses (Rosenberg et al. 2019).

In accordance with Wei et al. (2018) the good bacteria (Bifido species, including *B. longum* and *B. breve*) enhanced anticancer effects by blocking tumor progression when combining with immune treatment (Wei et al. 2018; Galdeano et al. 2019). A study confirmed that the probiotic (*C. butyricum*) supplementation in lung cancer patients decreased the systemic inflammatory response, reduced chemotherapy-induced diarrhea, and maintained intestinal flora condition (Tian et al. 2019). Some studies suggested that the administration of certain *Bifidobacterium* and *Lactobacillus* strains may reduce the risk of malignancy (Fooks and Gibson 2002; Tareb et al. 2013). Other *Lactobacillus* (An and Ha 2016), *Bifidobacterium* (Sivan et al. 2015), and Bacteroides strains (Vetizou et al. 2015) emerge to play significant roles in the efficacy of cytotoxic and immune therapies. Hendler and Zhang (2018) evaluated that the gut microbiome have a good connection between pathogenesis and treatment of colorectal cancer. Russo et al. (2007) and Orlando et al. (2012) stated probiotic *Bifidobacterium adolescentis and L. rhamnosus* strain exhibited significant anticancer effects against different types of tumor cells (HT-29, SW 480, Caco-2), and human gastric cancer cells. Moreover, according to Ghoneum and Gimzewski (2014) *L. kefiri* produces a cytotoxic effect against myeloid leukemia cell lines. The *L. lactis* clinically isolated from human GI flora significantly reduces the expansion and viability of various harmful bacteria in cancer cells, like MCF-7, HeLa, AGS, Caco-2, and HT-29 denoting their potential therapeutic role in cancer (Nami et al. 2015). Through in vitro and in vivo clinical studies it was confirmed that the potential healing effect of probiotics improved the immune system and regulated gut inflammation and bind with the toxic compounds present in the gut. So, it reduces the incidence of infections as well as resistance, mentioned in (Fig. 4.1). Maleki et al. (2015) suggested that the ingestion of *Bifidobacterium* or *Lactobacillus* at the doses of 10^{10}–10^{11} cfu/day for 4–6 weeks reduces the prevalence of cancer.

The function of healthy food is to give nutrition to convene an organism's functional necessities. The idea of a purposeful diet progressed lately after widespread investigation on the effect of a healthy diet on healthiness (Gibson 2007). Foods which are functional include certain constituents that offer health reimbursement by disturbing one or additional body function in a beleaguered way (Roberfroid 1999). Food supplements *or* efficient foods are composed of micronutrients, vitamins, antioxidants, biologically active peptides, and unsaturated fatty acids. Some dietary macro constituents from living microbes or plant chemicals have certain nutritional value but are not considered very essential. The frequently used nutritional technique to influence the flora of the gut is called probiotics (Brink et al. 2005).

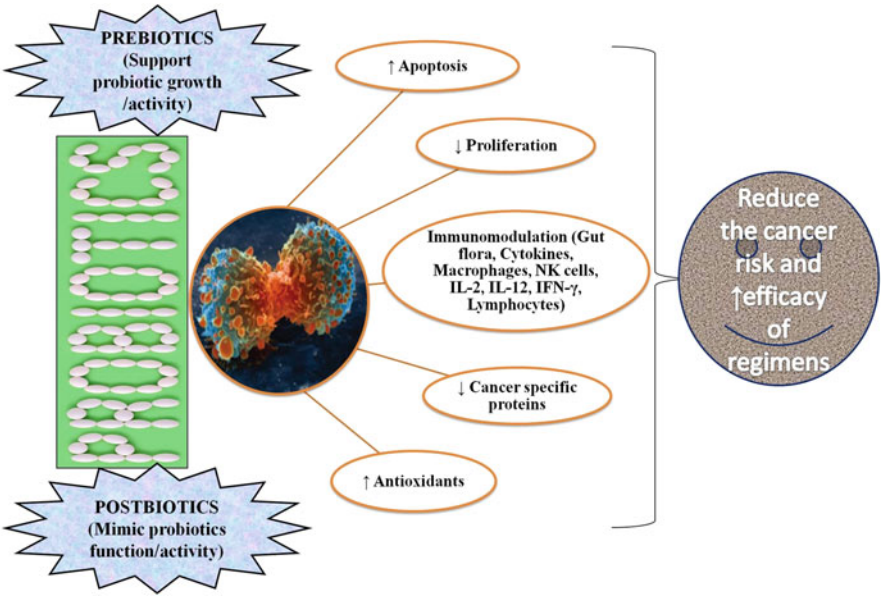

Fig. 4.1 Mechanism offered by probiotics in the prevention and control of cancer

Some issues of probiotic functionality correlated to instantaneous consideration for in-depth planning in terms of efficacy and safety, established their health claims alongside precise conditions in the target subjects (both in vitro and in vivo models). Their way of action and validation still intend clinical studies. Further, there are some more issues with probiotic, which include, their effective dosage, dispensation technique, and regulatory issues. Addressing these issues holistically through mutual discussions with all the alarmed stakeholders might pave the way in mounting a road map for probiotics for the welfare of the society (Smolin and Grosvenor 2000).

Generally, probiotics consists of live microorganisms mostly bacteria that are comparable to favorable microorganisms that originated in the human flora. Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (Saunier and Doré 2002). More than 10^{14} bacteria live inside the human gut, out of which some are reactive and few are dangerous. The degraded products of responsive bacterium (probiotic) are acetic acid and lactic acid; these can hold back the expansion of dangerous bacterium and maintain the wellbeing of the host (Harbolic 2012). Probiotics are administered for their assumed beneficial effect like detoxification and improvement in immune reaction (Medici et al. 2004; Ghafoor et al. 2005), antimutagenic and anticarcinogenic activities (Boutron-Ruault 2007; Davis and Milner 2009; Liong 2008) reduction in cholesterol levels (Zhang et al. 2008), antidiarrheal (Sampalis et al. 2010), alleviation of lactose intolerance (Guarner et al. 2005) and in

inflammatory bowel disorder (Kruis et al. 2004), as vitamin B supplement (Jamaly et al. 2011; Kneifel et al. 1992), and as an alternative and adjuvant with conventional Allopathic medicine (CAM) (Saarela et al. 2000). Patrons must be given with a sovereign appraisal of biological, microbial, and defense sorts of these live microbiome, in particular, if they can perquisite up healthiness. The most recent drift in the handy marketed nutrient is to merge both *probiotics and prebiotics* to increase the overall health beneficial effect of probiotics (Menrad 2003).

Prebiotics can define as "non digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of bacteria in the colon" (Gibson 2007). Probiotic trial should make use of the top practices accessible. If probiotics have to exert helpful properties, they have to be present in the viable form in the product and then later get themselves established in the gut flora. Potential diversity is found among probiotics like *Enterococcus faecium* and *E. faecalis* (Roberfroid 1999; Menrad 2003). The prescription of these useful foods needs careful monitoring (Alcid et al. 1994). To assure safety and functions the producer desires to be specific in the uniqueness of the probiotic strains (Roberfroid 2001). Globally both pre and probiotic product market is growing rapidly. Even though in evolving countries, it has sufficient opportunity and capability but simultaneously there is a rising need to stipulate it is used according to the health regulatory system. One of the important roles of the health care industries is to understand its significance in purposeful diet and in the various formulation including capsule. All the way through the market appraisal, several types of nutrients were found to contain Probiotics combination and prebiotics. Amidst them, few are refreshed baby foods, milk products, while little foodstuff supplements were established in India comprising merely probiotics. Probiotic having high viability and existence in the formulation and in the gut could be useful (Collins and Gibson 1999). Therefore estimation of the label claim holds a great importance for such products.

To achieve the therapeutic benefit, bacteria in probiotics have to be live and present in high concentrations, normally 10^7–10^8 cfu/g (Shah et al. 2000). In this study, it was established that living cells of all food supplements were 3–4 log cycles lesser than the claimed value and out of 4, 3 formulations are slightly effective for their projected wellbeing benefit. In diverse populations, apart from the significance of viability, a lot of surveys carried out to authorize sustainability and claims revealed that small number of probiotic bacteria are actually present in probiotic formulations (Shah et al. 1995). In vivo evaluation should be done for authentication of the efficacy of probiotics. Till now there is no directive for probiotic products. The customers are influenced via the claims made by manufacturers and vendors under deceptive right. The health claims on probiotics formulation should be provided to the customer with reliable evidences as these claims impact customer activities and possibly disturb community health (Clydesdale 1997). Many aspects can be held accountable for the reduction of the viability of probiotic microorganism, which include their acidic nature, production of acid during storage, the oxygen level in product, and their sensitivity to antibacterial substances (Dave and Shah 1997). Thorough investigation regarding this issue needs to be done on the priority basis.

4.2 Classification of Probiotics

Prebiotics are nutritional substances typically containing polysaccharides and oligosaccharides without starch, further inadequately digested by certain specific enzymes. They usually promote the growth of helpful bacteria over that of injurious ones. Normally prebiotics contains carbohydrates like oligo-fructose, galactooligosaccharides, lactulose, and inulin. Out of these lactulose is an artificial carbohydrate medicinally effective for hepatic encephalopathy and constipation. Furthermore, oligo-fructose can be synthesized either from sucrose by certain enzymatic reactions or obtained naturally from chicory roots, and present in various foods, such as honey, wheat, and in fruits and vegetables like in bananas, onions, and garlic. Oligo-fructose can be fermented in the colon and can alter the physiology of GIT.

Few of them are described as

- ↑Number of beneficial bacteria in the colon
- ↑ Absorption of calcium
- ↑ Fecal mass
- ↓ Time of gastrointestinal passage
- ↓ Lipid levels in blood

The role of prebiotics is to boost up numbers of cocci, by reducing the level of ammonia. This will eventually inhibit the growth of harmful pathogens. Prebiotics and Probiotics together offers symbiotic environment. Probiotics play an important role in increasing the production of gastrointestinal enzymes and vitamins.

4.2.1 Strains/Genera/Species

The study of Probiotics recommends a variety of impending payback toward health. However, the properties defined can be robust well into a strain but neither to a group or species of probiotics.

In technical society, taxonomically probiotics having different strains (Table 4.1).

4.2.1.1 *Lactobacillus Species*

There are about 100 species of *Lactobacilli* that have been identified. They are ubiquitous gram-positive, catalase-negative, spore-forming, fermentative, chemoorganotrophic, and microaerophilic, appear as cocci, bacilli, or rods. They are helpful in the digestion of protein, carbohydrate and in the breakdown of bile salts, further helps in synthesizing vitamin K and B inside the host cell. These bacteria are found in certain fermented foods and yogurt (Reddy 2006; Meurman 2005).

Table 4.1 Probiotic strains in harvest strain (substitute designations)

Sl. No.	Strain type	Trade name	Manufacturer
1.	*B. animalis DN 173 010*	Activia	Danone/Dannon
2.	*B. animalis subsp. lactis Bb-12*	Chr. Hansen	Danone/Dannon
3.	*B. breve Yakult*	Bifiene	Yakult
4.	*B. infantis 35624*	Align	Procter & Gamble
5.	*B. lactis HN019 (DR10)*	Howaru	Danisco
6.	*B. longum BB536*	Bifido	Morinaga Milk Industry
7.	*Enterococcus LAB SF 68*	Bioflorin	Cerbios-Pharma
8.	*E. coli Nissle 1917*	Mutaflor	Ardeypharm
9.	*L. acidophilus LA-5*		Chr. Hansen
10.	*L. acidophilus NCFM*		Danisco
11.	*L. casei DN-114 001*	Actimel	Danone
12.	*L. casei F19*	Cultura	Arla Foods
13.	*L. casei Shirota*	Yakult	Yakult
14.	*L. johnsonii La1 (Lj1)*	LC1	Nestlé
15.	*L. plantarum 299V*	GoodBelly	NextFoodsProbi
16.	*L. reuteri DSM 17938*	*L. reuteri* Protectis	BioGaia
17.	*L. rhamnosus ATCC 53013 (LGG)*	Vifit and others	Valio
18.	*L. rhamnosus LB21*	Verum	Norrmejerier
19.	*Saccharomyces cerevisiae (boulardii)*	DiarSafe, Ultralevure, etc.	Wren Laboratories, Biocodex, etc.
20.	*L. acidophilus* CL1285 and *L. casei* Lbc80r	Bio K+	Bio K+ International
21.	*L. rhamnosus* GR-1 and *L. reuteri* RC14	FemDophilus	Chr. Hansen
22.	VSL#3 (mixture of one strain of *Streptococcus thermophilus*, four *Lactobacillus* spp., and three *Bifidobacterium* spp. strains	VSL#3	Sigma-Tau Pharmaceuticals, Inc

4.2.1.2 *Bifidobacterium* Species

There are about 30 species of rod-shaped, anaerobic bacteria. Bifidobacteria, predominantly present in the large intestine. They generate lactic ions from the metabolism of lactose and also help in the synthesis of vitamins. They produce beneficial short-chain fatty acids from fermented indigestible carbohydrates (Galdeano et al. 2007; De Roos and Katan 2000).

4.2.1.3 *Streptococcus thermophilus* and *Lactobacillus bulgaricus*

These groups of microbes are used in yogurt manufacturing. They are found in the intestinal tract; produce large quantities of lactase enzyme for metabolism of lactose,

extremely useful for prevention and improvement of lactose intolerance, and have antimicrobial activity (Soccol et al. 2010).

4.2.1.4 *Saccharomyces boulardii*

This is the lactic acid-producing non-colonizing yeast useful as probiotic. They secrete proteases that break down microbial enterotoxins and inhibited their attachment to intestinal receptors. These are useful in the synthesis of vitamins and are able to trim down the cholesterol level in serum. They are useful for the prevention and treatment of traveler's and antibiotic-associated diarrhea occurred due to the *C. difficile* infection. Further, it can be used for the treatment of acne and can reduce the side effects associated with *H. pylori* treatment (Saraf et al. 2010).

The usual communications amongst human gut microbiota with their group maintain a symbiotic connection. Large proportions of small-intestinal lymphoid cells have an important effect on the immune system as well as on upper intestinal bacterial load. These epithelial cells act as an accepter of antigens, further lymphoid germinal cores initiate the process of adaptive immunological responses. Microbes can replicate by fermentation inside the colon around substrates either from food or endogenous secretions. In the intestine about 60% of total immune cells are present. Normally probiotics influence duodenal bacteria by diminishing the number of potentially pathogenic microbes, simultaneously increasing the amounts of useful anaerobic bacteria. The Probiotics upset the intestinal bio-network by exciting immune mechanisms through encouraging nonimmune mechanisms and antagonizes the effect of potential pathogens. These phenomena provides an idea to facilitate a few helpful things, which include a decrease in the frequency of diarrhea, which is one of the widely familiar usages for probiotics. It reduces the risk of colon carcinoma in animal models, most likely as a result of their function in subduing the activity of definite bacterial enzymes that may add to the quantity of pro-carcinogens, however, this has not been confirmed in humans yet (Fig. 4.2).

4.3 Potential Benefits of Probiotics in Cancer

Probiotics are known to colonize, reproduce, and make a range of biologically active materials known as metabiotics which play a role in digestive tract disorder. They enhance ionic balance in the individual GIT and prevent the exchange of pro-carcinogens to the carcinogenic product by declining certain destructive enzymes such as beta-glucuronidase, nitroreductase, and beta-glucosidase (Verma and Shukla 2013). Also short-chain fatty acids (SCFAs) are reported to increase the levels of an enzyme called Glutathione S-transferase (Scharlau et al. 2009) and pass on genetic constancy to the colon cells. Fermentation of high amylose starch form butyric acid is known to decrease the oxidative reactions in the gut and sometimes trigger diverse pro-carcinogen bio-transforming enzymes to support the prevention of colon carcinoma (Clarke et al. 2011). Liong (2008) reported that the presence of SCFAs can change the state of a cancer cell from apoptosis to the necrosis in a reduced environmental pH created by them. The study revealed that the presence of

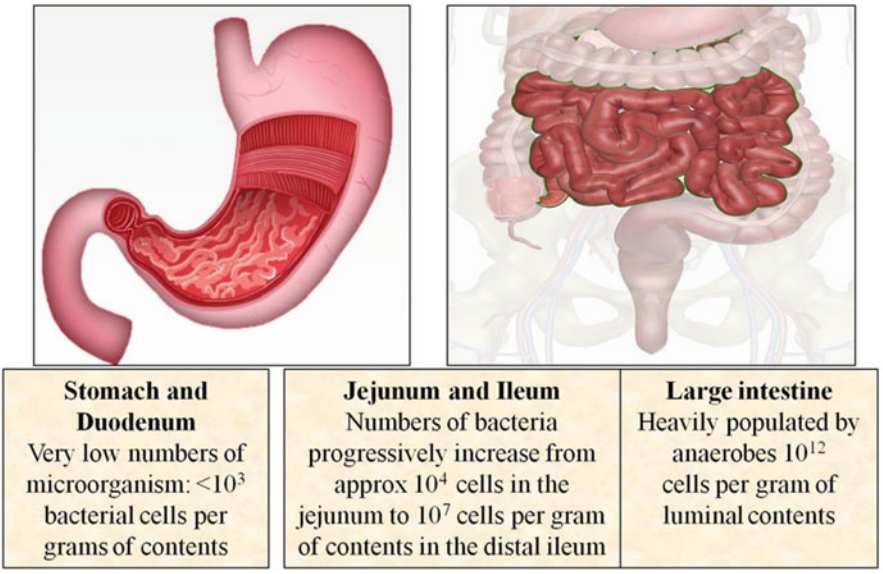

Fig. 4.2 Sites of the existence of probiotics microorganism in GIT

Bifidobacterium reduced the cancer-causing property of a carcinogen azoxymethane and thus play a role in decreasing the incidences of colon carcinoma.

4.4 Mode of Action of Probiotics

Probiotics actively participated in the fermentation of nondigestible substances, and are recognized as prebiotics, for their vigor and wield selected property like against pathogenicity, obesity, diabeties, inflammation, cancer, and other angiogenic effects and in the brain and central nervous system disorders (Kerry et al. 2018). On the other hand, the purpose of probiotics can be categorized as metabolic, defensive, and trophic (Kusku-Kiraz et al. 2018). The main important pathway of intestinal microbes is in cancer therapy. Disturbed gut biosis have been held responsible for the reduction of the immune response which could lead to tumor resistance for drugs like cyclophosphamide (Viaud et al. 2013) or oxaliplatin (Iida et al. 2013). Reports highlight that probiotic bacteria can acts as a constituent in cancer immunotherapy regimen (Pitt et al. 2016; Poutahidis et al. 2014; West and Powrie 2015; Wan and El-Nezami 2018). The mechanism of probiotic action can be listed as under.

- Rivalry for binding sites—Helpful bacteria can adhere to the intestinal wall and produce colonies at several places through the gut. This prevents pathogenic/infectious bacterium from establishing themselves, thus, leads to their removal from the body.

- Increase in digestion—Probiotics have found immense usefulness in improving the host digestion.
- Lactic acid creation—Probiotics yield lactic acid which result in the reduction of gut pH that bars the growth of pathogenic bacteria, which generally prefer an additional alkaline atmosphere.
- Influence on immunity—Probiotics have been revealed to increase the stages of cell signaling events and the power of infection-fighting cells (W.B.C). Further, the effectiveness of probiotics rests on several factors like dose administration, mode of administration, strains, duration of treatment, and health status of individual (Holzapfel et al. 1998).
- Regulating intestinal ionic balance—Probiotic bacterium plays a vital function in the colon by safeguarding the ionic balance and by maintaining normal physiological conditions.
- Other effects—Probiotic microbes are also accountable for possessing the symmetry between the quantity of added members of normal intestinal microflora and their action. Putrefactive microbes present in nature also exist in gut flora and were proved to be responsible for the generation of carcinogens by enzymes similar to beta-glucuronidase, and reductases. Some initial discovery by Goldin and Gorbach in 1970s confirmed that intake of fermented milk has positive effect on raising the number of *L. acidophilus* in the gut in the rat model, which consequently cause a drop in number of putrefactive microbes and reduced harmful enzymes level (Wan and El-Nezami 2018).

Numerous literature reports confirmed about the efficacy of the probiotic strains against bacterial enzymes drawn from a tumorigenic study in rodents (Rowland et al. 1998) and human (Borruel et al. 2002; Savan and Sakai 2006; Reddy et al. 1977). Data from nitroreductases and glucuronidase reports are ordinarily reliable but still, whether these techniques able to reduce cancer incidences in humans is yet to be explored (Goldin and Gorbach 1976). Wan and El-Nezami (2018) evaluated nineteen case studies and reported that a connection between the consumption of milk products (other than cheese) can lead to reduced cancer threat. Another striking move toward identifying the potency of milk foodstuffs on post-indicative CRC was reported by Rowland et al. (1998).

Numerous mechanisms are involved in the protective role of probiotics against colorectal cancer inception. It comprises modification of the gastrointestinal microbes and inactivation of cancer-causing substance; fight with putrefactive and disease-producing microbes; development of the host's immunity; and antiproliferative effects via apoptosis and cell differentiation. The following sections will address every mechanism in detail.

4.4.1 Altered Metabolism in Intestine by Altering Intestinal Microflora

Metabolism occurs in our body by two pathways—Phase I and Phase II reactions. Through the conjugation reaction of Phase I, most of the harmful chemicals, i.e., both endogenous and exogenous including carcinogens, are metabolized to polar nontoxic compounds, which are easily excreted from the body through bile (Rowland et al. 1998). Intestinal bacterial β-glucuronidase causes deconjugation of the formed glucuronides (products of phase I metabolism) and form aglycones, which are principally cancer-causing substances. Loads of bacterial enzymes present in stools, such as azo-reductase and nitroreductase, can minimize the generation of procarcinogenic materials in the intestine (Hill 1975; Goldin and Gorbach 1976). So probiotics could alter the concentration of the bacterial enzymes, which are responsible for the generation of pro-carcinogens. Interfering with carcinogenic metabolism might be the possible ways by which probiotics may reduce the risk of onset. The research evidence supported the same in preclinical studies and showed that reduced levels of β-glucuronidase and nitroreductase were found in the large intestine of probiotic (*L. acidophilus)* treated colon carcinoma mice.

4.4.2 Inactivation of Carcinogens Produced in GIT from Diet

The generation of carcinogenic products inside the intestine is mainly found in Red Meat in the form of heterocyclic aromatic amines (HAA). It is degraded into powerful mutagenic substances when it comes in contact with gastric microbiota. Such influential substances may turn together with the colonic mucosa and thereby causing cancer (Wakabayashi et al. 1992). LAB and additional commercial bacteria have been bringing into to bind or break down some carcinogens (Kumar et al. 2010).

Causes

There are certain factors that can affect probiotic action. The likeness to connect and metabolize toxic substances depends on hydrogen ion concentration and other physiochemical situation. The entire outcomes specify that food mutagenic compounds, commonly found in processed meat, are detoxed. This can antagonize the onset of colorectal cancer (CRC). Further, animal protein also increases the H_2S level, which is recognized to be genotoxic experimentally. It is well known that a diet rich in animal meat excites the expansion of bile salt-forming bacterium and has confirmed cytotoxicity and carcinogenicity (Kovac et al. 2018; Uccello et al. 2012). Few of the examples of probiotic strains and their applications in cancer therapy are mentioned in Table 4.2.

Putrefactive intestinal microbiota are mainly responsible for colorectal cancer like Bactericides species and Clostridium species. On the other hand, several LAB species possess preventive attributes against cancer (Sobhani et al. 2011). Clinical

Table 4.2 Probiotics application in cancer therapy

Name of organism	Types of enzymes modify or produce	Types of cancer
Bacteroides spp	–	Colorectal cancer
Clostridium spp	–	Colon cancer
Lactobacillus casei Shirota	Interferon-γ, interleukin-β, and tumor necrosis factor-α	Bladder cancer
Saccharomyces boulardii	Epidermal growth factor receptor [EGFR]	1. Intestinal tumor 2. Human gastric ulcer/ disorders
Lactobacillus reuteri	↑Proapoptotic mitogen-activated protein kinase	Colon cancer
Lactobacillus casei	↑Cytokines, such as interferon-γ, interleukin-β, and TNF-α	Human bladder cancer cells

isolates from the stools of colon cancer patients, to whom symbiotic combination of inulin enriched with specific oligo-fructose were given, showed significantly decreased levels of *Clostridium perfringens* (O'mahony et al. 2001).

4.4.3 Boosting Host Immunity

Probiotics were described to correlate with entrecotes, dendritic, Th1, Th2, and Treg cells of the large intestine and revise the acquired immunity into pro and antinociceptive action. Probiotics basically produce two types of action toward immunity, i.e., immunostimulatory and immunoregulatory. The immunostimulatory probiotics are mainly useful in disorders like cancer, an allergic reaction, and for other infections, whereas immunoregulatory probiotics are beneficial for autoimmune disorders, irritable bowel syndrome, and other allergic reactions (Azad et al. 2018). The immunomodulatory outcome of probiotics generated cytokines, jointly with interleukins (ILs), tumor necrosis factors (TNFs), interferon (IFNs), transforming growth factor (TGF), and moreover SCFAs. Furthermore, other bioactive factors obtained were EPSs, solitary coating proteins, peptidoglycan, lipoteichoic acids, conjugated linoleic acids, and peptides. These factors strengthen the immune system and even heat-killed probiotics are valuable in prevention against cancer and improvement of the immune system owing to the presence of these factors (Sekine et al. 1985a, b). Among them, particularly *Lactobacillus Casei Shirota* is highly effective in colon cancer not due to its direct toxic effect but for its enhanced regulatory effect on host immunity as evaluated in different experimental models (Kato et al. 1981).

4.4.4 Producing Antiproliferative and Cytoprotective Effect by Apoptosis

Cancer and tenderness are matching one another and the connection of it was experienced more than a hundred years back, for the first time by Virchow, from the occurrence of leukocytes in neoplastic cells (Francescone et al. 2014). More than 20–25% of carcinoma, showed chronic unrestrained inflammation, which is involved in the commencement and development of tumor e.g., rabble-rousing from bowel disorder development to colon carcinoma. Further, hepatitis leads to HCC and *H. pylori*-induced gastritis to gastric carcinoma. Long-lasting and uncontrolled inflammation enable cancer development (Sharma and Shukla 2016). Butyrate is a major probiotic metabolite useful in Colon cancer due to its inhibitory action against HDAC with acting as a regulator of apoptosis with enhanced intestinal epithelial barricading the integrity by calculating the occludin and cingulin connection proteins (Bordin et al. 2004). Probiotics can have regulatory effect on apoptosis as well as in cell proliferation. This could affect their concentration-response curve (Iyer et al. 2008). As reported earlier *Lactobacillus reuteri* reduced TNF production and showed a gradual increase in dose response by accelerated apoptosis especially in activated immune cells. This leads to the management of irregular cell proliferation via reticence of IkB ubiquitin and striking cell signaling. Patel et al. (2012) confirmed the same in mice.

Besides butyrate, other SCFAs like acetate and propionate have reported better apoptotic potency by generating ROS, caspase-3, and distressing the mitochondrial transmembrane potential, followed by nuclear chromatin abridgment in colon cancer cells (Lan et al. 2007). These SCFAs (propionate and acetate) are conversely requisite in higher concentrations to persuade apoptosis and show diverse metabolic effects compared with butyrate (Hosseini et al. 2011). Propionate too inhibits cancer occurrence as it can be transported easily to the liver through the portal circulation. Unpredictably, butyrate and propionate together originate to fetch on autophagy rather than apoptosis in cancer cells due to some mitochondrial defects, revealing the reason behind mixed results observed with SCFA treatment (Bindels et al. 2012).

4.4.5 Fermentation of Unabsorbed Food by Microorganism

The undigested carbohydrates are metabolized by bacteria to produce short-chain fatty acids (SCFA) and gas. The gas was removed and the SCFA (commonly acetate, propionate, and butyrate) stand for nutrients and develop certain signals which may play a role in CRC prevention for the intestinal mucosa (Mai 2004).

4.4.6 Regulating Signaling Pathways

These pathways are activated by receptors or cytoplasmic proteins with active tyrosine kinase and play a critical role in carcinogenesis (Lemmon and Schlessinger 2010). *Saccharomyces boulardii* is a safe probiotic mediator used extensively to prevent or treat a wide selection of human GI disorders. It has been reported that *Saccharomyces boulardii* acts from side to side variation of the host signaling pathways that switch the intestinal mucosal inflammatory response (Sullivan and Nord 2002). *Saccharomyces boulardii* down-controls MAPK signaling pathways that causes downstream of many growth factor receptors and epidermal growth factor receptor (EGFR). The EGF receptor family contains four members: ErbB1/EGFR/HER1, ErbB2/HER2/NE, ErbB3/ HER-3, and ErbB4/HER-4 that are significant for cancer growth (Hynes and MacDonald 2009). *Saccharomyces boulardii* was found to prohibit cancer cell colonization, reduce EGF mediated apoptosis, and cell proliferation (Chen et al. 2009). Both in vitro and in vivo findings were consistent for involvement of the EGFR and Akt pathways. Ma et al. (2010) verified that the probiotic *Bacillus polyfermenticus* reduces tumor growth in vivo and sheltered colon cancer cells expansion in vitro.

RAS oncogene activation is the earliest and most frequent genetic alteration seen in colon cancer, cell proliferation, nuclear aplasia. *Bifidobacterium longum* administration is reported to suppress the colonic mucosal P-21 expression in tumors as compared to the control diet. Further, it was found to acts as an immunomodulatory and modifier of biological reaction in tumor suppression (Azad et al. 2018).

4.5 Safety and Risk Factors About Probiotics in Clinical Practice

Probiotics are synchronized as nutritional supplements other than any medicinal product. In general, safety, purity and potency are not obligatory requisites before promoting probiotics. For which, significant irregularities arise between the actual standard and adulterated stuffing of probiotic preparations, as reported from a study from South African (Theunissen). According to Europe, food supplements used by infants and juvenile children should possess time dropper compositional requirements. While in the USA, biological foodstuffs useful for various disorders should be reviewed by the Food and Drug Administration for the consent for their marketing. Likewise, in Australia, probiotics available for good health need premarket appraisal from the Health & Administration officials and are generally known as matching therapeutics. While in the case of Japan, a proper premarket evaluation report by the Health Ministry is required for probiotic foodstuffs meant for a specific health issue. Even though, the majority of commercially obtainable probiotic strains are generally considered as safe still there are considerable concerns that exist with reverence to their safety for clinical application.

4.5.1 Risk Factors

Various risk factors like infection and sepsis are reported on clinical applications of probiotics. Rautio et al. (1999) reported that a 74-year-old diabetic woman built-up hepatic eruption and pneumonia after 4 months of therapy of LGG supplementation daily. While in another research Mackay et al. (1999) observed that the administration of *L. rhamnosus* leads to the progress of endocarditis infection in a 67-year-old patient.

Generally, probiotics derived from bacteria or fungi do not produce any kind of sepsis when given to immune-compromised patients having any chronic disorder. However, if some sepsis arises on its administration it can be controlled with certain antimicrobials. Though in few patients septic shock persists and can be fatal. The cause behind such cases is generally some underlying preexisting conditions (Rijnders et al. 2000). Lestin et al. (2003) reported that a 48-year-old diabetic female with *Clostridium difficile* attributed diarrhea with septic shock in alliance with a toxic megacolon and probiotic fungemia died from multi-organ failure. The superior susceptibility of quick response from infants as well as from immune compromised to probiotic sepsis could be obtained from preclinical findings (Wagner et al. 1997).

4.5.2 Deleterious Metabolic Activities

The Intestinal microbiota plays a vital role in numerous metabolic actions, including lipid metabolism, glucose homeostasis, and complex carbohydrate digestion. Therefore it is considered that by altering the microbiota, the risk of deleterious functions can be addressed (Saavedra et al. 2004).

4.5.3 Immune System Depression or Hyperstimulation

From the preclinical evidences it was revealed that the gut microbiota showed significant improvement in the development of the immune system, mainly the progress of gut-associated lymphoid tissue. The gut microbiota is required for a sequence of immunological actions, and the development of germinal centers in lymphoid follicles (Backhed et al. 2005).

The long-term effect of certain alterations on the host is hard to envisage and reports claim that probiotic supplementation may cause medium or long-term alteration in the microbiota or would cause lifetime alteration of the immune system. Probiotic supplementation to pregnant women can lead to adverse immune stimulation. It was found that in pregnancy T cell responses were altered and Th1 cytokines may be held responsible for miscarriages (Wegmann et al. 1993). *Lactobacillus* was found to stifle Th2 cytokine response in humans, which may pose a high risk of expecting women (Pohjavuori et al. 2004). However, a detailed examination is required.

4.5.4 Antimicrobial Resistance

The research reports confirm that probiotics colonize in the host intestine rapidly. On the other hand, the interesting thing is the likely shift of the antimicrobial battle of probiotic to pathogenic bacteria in the intestinal flora. Majority of *Lactobacillus* are defiant to vancomycin, it raises worries concerning the likely allocation of resistance to further pathogenic organisms, mainly *Enterococci* and *S. aureus*. Vancomycin-resistant genes are present in *Lactobacillus*, however, such genes are missing in other genera (Tynkkynen et al. 1998).

4.5.5 Specificity of Probiotics Effects

The medicinal or preclinical application of one probiotic cannot be implied for one additional probiotic category or dissimilar strains of the same species. The deviation in properties is possible to cause strain-to-strain disparity in both microbiology and pathogenicity. Wagner et al. (1997) reported the property of four diverse probiotic species (*L. reuteri*, *L. acidophilus*, LGG, and *B. animalis*) in preventing colonization and sepsis in both athymic and euthymic mice while infected with *Candida albicans*. It established that all strains were defensive, but there were noteworthy differences in their effectiveness and produces variable immunological responses in terms of proliferation and antibody production to *C. albicans* gastrointestinal provocative cell infiltration. Further, the in vitro study confirms the variety of actions of diverse probiotics (Wagner et al. 1997). Another report revealed that different strains of the same probiotic can possess opposite effects. Two different strains of *Lactobacillus* (*L. reuteri* DSM12246 and *L. casei* CHCC3139) were investigated for dendritic cell role and it was found that only *L. casei* CHCC3139 was able to induce interleukin (IL)-12, IL-6, and tumor necrosis factor production (Christensen et al. 2002). Similarly, study of the *Bifidobacterium* species on the dendritic cell has shown a noticeable difference between species (Young et al. 2004). Clinical findings confirmed the implication of both in vivo and in vitro results. Furthermore, Allen et al. (2003) reported a mixture of *L. acidophilus* and *L. bifidus* was mainly effective for improving the production of interleukins. It is therefore very important to review a probiotic strain for its clinical effectiveness. Nonetheless, in certain medical conditions, a series of diverse probiotics emerge to be effective apparently by performing a similar mechanism to an array of nonpathogenic microbes. Further research is required to confirm its importance of strain-specific property in diverse situations with probiotic–probiotic relations.

4.6 Other Clinical Applications

According to existing insight from the experimental research Fig. 4.3, includes updated clinical applications of probiotics.

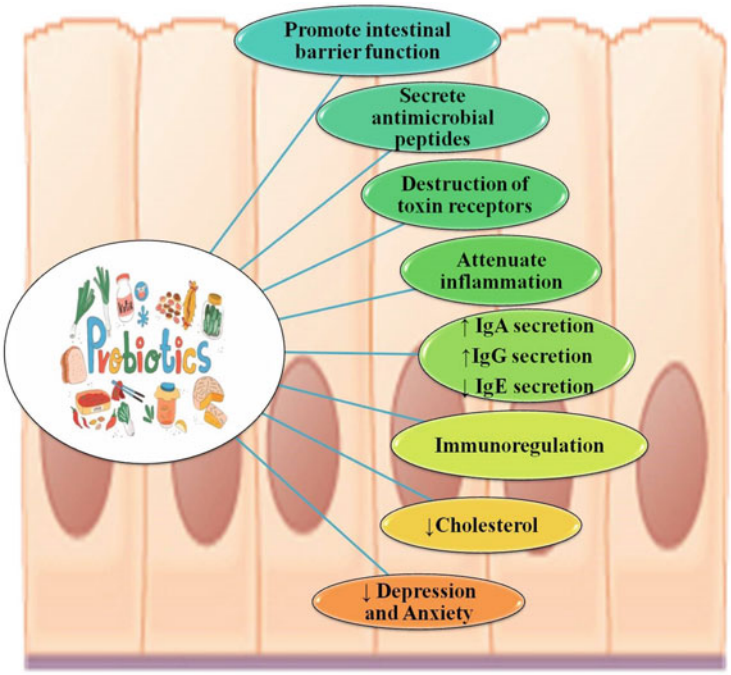

Fig. 4.3 Clinical application of probiotics

4.6.1 Diarrhea

4.6.1.1 Treatment of Acute Diarrhea

There were some evidences reporting applications of probiotics in combating the conditions of acute diarrhea by reducing the frequency of defecation in children, upon oral administration. Furthermore, probiotics were widely effective in viral gastroenteritis as compared to parasitic and bacterial dysentery. Some highly effective strains are *L. reuteri* ATCC 55730, *L. casei* DN-114 001, *L. rhamnosus* GG, *Saccharomyces cerevisiae*, and *S. boulardii*.

4.6.1.2 Antibiotic-Associated Diarrhea

Prolonged antibiotic therapy usually causes diarrhea. Few attempts were made to test the usefulness of probiotics to control diarrhea in both adults and children along with usual antibiotic therapy. Effective probiotic strains are *S. boulardii*, *L. casei* DN-114 001, and *L. rhamnosus*.

4.6.1.3 Radiation-Associated Diarrhea

Application of probiotics in the therapy of radiation-associated diarrhea was not much explored, but still there were certain evidences that report efficacy of probiotic strains like *L. casei*, *L. acidophilus*, *L. plantarum*, *L. delbrueckii*, *Bifidobacterium*

infantis, *B. breve*, *and Streptococcus thermophilus* in the therapy against radiation-associated diarrhea.

4.6.2 Eradication of *Helicobacter pylori*

According to evidences lactobacilli, *Bacillus clausii*, and few bifidobacterial strains are useful in minimizing the adverse responses of antimicrobials with improved patient acquiescence. Few of them are responsible for reducing the adverse effects but do not possess any therapeutic effect. Randomized clinical trials confirmed that probiotic therapy along with prokinetic agents improved the rate of eradication of *Helicobacter pylori*. Although sufficient research is yet to be done to confirm probiotics potency as an Anti-Helicobacter pylori agent, the literature suggests that certain probiotics are useful as additive therapy along with antibiotics for combating *H. pylori* infection.

4.6.3 Allergy

It has been mentioned in some reports that probiotics are helpful in preventing certain type of dermatitis conditions if given to pregnant women in addition to neonates up to 6 months, yet no such clinical evidences are present. With gaze to this dealing of allergic conditions, some ingenious research reported facts that unambiguous probiotic strains are successful in the therapy of few patients having atopic dermatitis.

4.6.4 Liver Encephalopathy

According to evidences, prebiotics (mainly lactulose) are generally considered as a second-line agent for the anticipation and therapy for progressive hepatic cirrhosis. Clinical study highlight that, liver enlargement was upturned in 50% of patients treated with a symbiotic homework (4 fermentable fibers and four probiotic strains), like inulin and starch when used for 30 days.

4.6.5 Inflammatory Bowel Disease (IBD)

4.6.5.1 Pouchitis

Some confirmed cases of the applications of probiotics in preventing an early attack of pouchitis was observed. Its introduction to therapy could reduce the uses of conventional antimicrobials.

4.6.5.2 Ulcerative Colitis

The probiotic combination was reported to show therapeutic potential in ulcerative colitis patients.

4.6.5.3 Megacolon Conditions

Megacolon conditions are also known as Cohn's disease. Reports regarding the usefulness of probiotics in the condition were unsatisfactory. Furthermore, no evidence supports either therapeutic or preventive application of probiotics in remission of Cohn's disease. Flatulence and bloating were reduced by probiotic uses. Recent clinical trial on ninety breastfed infants revealed that colic is a side effect of probiotic administration.

4.6.6 Lactose Intolerance

Administration of *L. delbrueckii*, *S. thermophiles* subspecies, and Bulgaricus can improve lactose absorption. This report was confirmed by many controlled trials where individuals were taking yogurt and live cultures together.

4.6.7 Necrotic Enterocolitis

From clinical research, it was confirmed that supplementation of probiotics decreases the chances of occurrence of necrotic enterocolitis in premature infants. Efficient reviews of randomized clinical reports have abridged the cases of fatality in probiotic given groups. The ratio of decreased fatality is 1:20, means 1 may die out of all 20 treated.

4.6.8 An Alcoholic Fatty Liver Disorder (AAFLD)

Till date, the supportive effect of probiotics as an alternative therapy has not been clinically established through sufficient randomized trials.

4.6.9 Preclusion of Systemic Infections

There are inadequate facts to claim the application of probiotics as well as symbiotic in seriously ill patients in intensive-care units.

4.7 Recent Advances with Future Prospective for Other Clinical Applications

The recombinant probiotics and genetic interactions among the native intestinal microbes and ingested probiotics can be a topic of interest. These modified microorganisms may have better antimicrobial activity with reducing antibiotic resistance, can be used as carrier for delivery of vaccines and therapeutics gene, and can be of great potential to encourage new approaches in prevention, control, and treatment of various ailments (Fig. 4.4).

4.8 Conclusion

In brief, this chapter describes about the future efficacy of probiotics in cancer either in the prevention or by controlling it through a different mechanism (mainly by enhancing hostile immunity it may alter gut inflammation caused by any toxic substance). Further, probiotics are responsible to improve immune cell proliferation

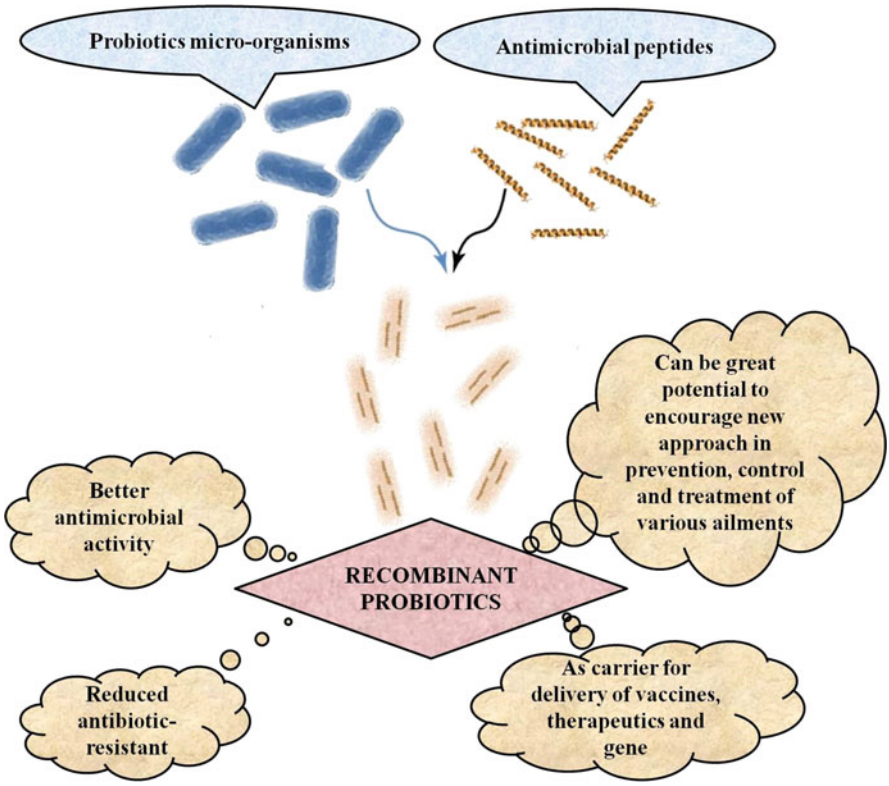

Fig. 4.4 Future prospective of probiotics

and enhance the production of inflammatory cytokines, TNF, and some important interleukins, depending upon the bacterial strain. Some specific probiotic bacteria hold the therapeutic potential to fight against the cancer occurrence or prevalence.

Probiotics containing food supplements and even fortified baby foods are liberally present in the market. No regulatory guidelines are available to control their quality in many countries including India. This enables the distributors to confuse the public with some sort of health claims, which is not exactly true. So this is the right time to create appropriate policy and guidelines to be followed before marketing either probiotics or prebiotics containing food products. Ultimately better quality pre/probiotic-rich functional foods could be produced and distributed for the sake of customers.

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Metabiotics in Colorectal Cancer: Crosstalk Between Gut Microbiota and Host Pathology

5

Monica Gulati, Sachin Kumar Singh, Rajesh Kumar, Kamal Dua, Simanchal Panda, James Blaxland, and Lipika Chandwani

Abstract

After an extensive research and successful commercialization of probiotics in the past three decades, some scepticism regarding their safety and reproducibility of therapeutic advantage paved the way for "metabiotics". The name metabiotics is a portmanteau created from the terms metabolites and probiotics. Also known as parabiotics and postbiotics, metabiotics are components of probiotic microorganisms and/or their metabolites with a determined chemical structure, which have been reported to be effective in the prevention and treatment of colorectal cancer (CRC). The main components of metabiotics are short-chain fatty acids (SCFAs), peptides, peptidoglycan-derived muropeptides, enzymes, polysaccharides, vitamins, teichoic acids, proteins and plasmalogens. Metabiotics help in the maintenance of GIT homeostasis and lead to proliferation of the healthy bacteria, which in turn reduces the levels of enzymes that are responsible for conversion of pro-carcinogens to carcinogens. Some components of metabiotics, specifically, SCFAs, have the ability to recognize cancer cells and de-repress the epigenetically silenced genes in them. The chemoprotective enzymes, secretory glycoproteins, certain exopolysaccharides and SCFAs all possess anti-mutagenic properties and exert a prophylactic effect against

M. Gulati (✉) · S. K. Singh · R. Kumar · S. Panda · L. Chandwani
School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, India
e-mail: monica.gulati@lpu.co.in; sachin.16030@lpu.co.in; rajesh.23035@lpu.co.in; simanchal.panda@pratap.edu.in

K. Dua
Discipline of Pharmacy, Graduate School of Health, University of Technology Sydney, Sydney, NSW, Australia
e-mail: kamal.dua@uts.edu.au

J. Blaxland
Cardiff Metropolitan University, Cardiff, UK
e-mail: jablaxland@cardiffmet.ac.uk

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colorectal cancer. Apart from this, these components regulate the immune function and downregulate the inflammatory mediators, the most prominent causative factor in the development of CRC. Metabiotics have also demonstrated the antiproliferative effects and have been reported to increase the gut membrane integrity. This chapter discusses the potential of metabiotics as an effective strategy for prophylaxis or therapeutic option in treatment of CRC.

Keywords

Metabiotics · Colorectal cancer · Short-chain fatty acids · Probiotics · Dysbiosis

5.1 Introduction

Eubiosis, the harmonic equilibrium not only among the various commensal bacteria, fungi and viruses but also between these members of microbiota, their metabolic products and the immune system of the host, is essential for the maintenance of healthy status in humans as well as animals. In contrast to the age old "sterile womb paradigm", it is now proven that a healthy prenatal microbiome is acquired in utero. Prenatal microbiome, in fact, is believed to play a significant role in the foetal development. The nature of gut microbiome in neonates, in fact, has been found to vary with the nature of delivery; natural vaginal birth leading to healthier gut microbiota as compared to that with caesarean section (Iebba et al. 2016).

Postnatal microbiome, in contrast, is influenced maximally by the feeding practices leading to normal or aberrant constitution of the gut microbiota. A great variation in the microbiome of bottle-fed and breast-fed babies has been reported. Breast feeding, specially the exclusive one, has been reported to exert strong protective effect against diarrhoea and long-term risk of carcinogenesis, diabetes and obesity as compared to bottle-feed, which is attributed to its effects on the infant gut microbiota (Mutic et al. 2017).

Manipulation of human microbiome and its metabolites has opened multiple avenues to modulate inflammatory responses, particularly in the areas of mucosal immunity and inflammation. A tailored interplay of dietary, probiotic, prebiotic, metabiotic and other microbiome-based therapeutics like faecal microbiota transplant (FMT) can be used to affect the gut immune system, including gut-associated lymphoid tissue, T helper cells, inducible regulatory T cells, IgA-producing B cells and innate lymphoid cells. Toll-like receptors (TLRs) that recognize the bacterial products like lipopolysaccharide, DNA and lipoteichoic acid and mediate protection from epithelial injury play a crucial role in the maintenance of intestinal epithelial homeostasis and modulate the activation of the immune system. Microbiome also supplies essential nutrients, modulates energy metabolism and participates in epithelial cell exfoliation. Restoration of the gut microbiome has been shown to be quite effective in treatment of various cardiometabolic disorders, inflammatory diseases, neuropsychiatric diseases and cancer (Wilkins et al. 2019).

In CRC, the gut microbiome and CRC are reported to display a bidirectional self-feeding relationship. Gut microbiota and host factors, like age and genetic predisposition, contribute to CRC progression. However, tumour outgrowth into gut lumen compromises the intestinal barrier, resulting in increased infiltration of gut microbiota into deeper tissue, leading to further stimulation of immuno-inflammatory response, which, in turn, further perturbs the gut microbiome (Gagliardi et al. 2018).

5.1.1 Role of Dysbiosis in Colorectal Cancer

The gut microbiome helps to maintain a homeostasis in the body. Any imbalance in normal microflora of colon has been reported to lead to colonic disorders leading eventually to the development of CRC (Han et al. 2018). In the colonic micro-ecosystem, there exists a dynamic equilibrium among colonic microbiota, mucosal epithelial cells, diet components that act as probiotics and prebiotics, enzymes, mucus and bile salts. Colon, being the main colonization site in the body, houses a large number of microbial cells (more than 1000 bacterial species). Bacteroidetes, Firmicutes, Actinobacteria, Verrucomicrobia and Proteobacteria are the dominant ones among them. Such unparalleled microbial colonization is attributed to a number of factors such as near neutral pH, low concentration of bile salts, anaerobic conditions, long transit time, high viscosity and very weak peristalsis prevalent in colonic milieu. Because of the action of the bacterial enzymes on the undigested dietary residues as well as endogenous mucins in the colon, the colonic microbiota acts like a metabolic organ (Tremaroli and Bäckhed 2012).

An optimal balance among various components of this micro-eco-system results in production of essential nutrients, their absorption, strengthening of the immune system and prevention of pathogen colonization. Dysbiosis, in contrast, leads to inflammation, damage of tissue mucosa and compromise in barrier integrity and function. All these factors alter the ratio of resident to potential oncopathogenic microbes leading to colonic oncogenesis. Bacteria with carcinogenic potential exert their effect by different mechanisms. *Fusobacterium nucleatum* and *Bacteroides fragilis* modify the E-cadherin/beta-catenin signalling, through their nuclear factor-kappa b (NF-kb) signalling pathway leading to inflammation (Rubinstein et al. 2013). *Escherichia coli*, however, produces enterobacterial genotoxins, which have high tumorigenic potential (Taieb et al. 2016). *E. faecalis* is reported to produce extracellular superoxide, which leads to oncogenesis attributable to DNA breaks (Boonanantanasarn et al. 2012). In fact the correlation between gut dysbiosis and CRC is so strong that non-invasive gut microbiome (GM) biomarkers are being proposed as screening biomarkers with high accuracy (Yang et al. 2020).

5.1.2 Metabiotics in CRC

Susceptibility of an individual to carcinogenesis, especially that leading to colorectal cancer (CRC), has been reported to be influenced significantly by the equilibrium between microbial production of metabolites such as short-chain fatty acids (SCFAs), some glycoproteins/peptides and potentially carcinogenic substances like amines and secondary bile acids. A single term used collectively for all these useful bioactive substances are referred as metabiotics (Sharma and Shukla 2016). However, the terms postbiotics, biogenics or simply metabolites/CFS (Cell free supernatants) are also interchangeably used. These include one or more components from the structural components of probiotics, their metabolites and signalling molecules. All these components possess a resolved chemical structure and are capable of contributing to the physiological functioning of host microbiota.

These low-molecular weight compounds (LMWs) are produced in the gut by the commensal microorganisms as well as administered probiotic strains by breaking down the nutrients and substances like saliva, components of gastrointestinal secretions, dead cells like microbes, epithelial cells etc. that originate from GIT itself. These include SCFAs, biosurfactants, polysaccharides, peptidoglycans, teichoic acids, lipo- and glycoproteins, vitamins, antioxidants, nucleic acids, coagulation factors, protein-like enzymes and lectins, peptides, amino acids, growth factors, defensin-like molecules or their inductors in human cells, signal molecules, plasmalogens etc. As different microbial strains produce different sets of LMWs, the larger the biodiversity of gut, the healthier would be the host gut physiology. A significant interplay between gut microbiota and host metabolism has been widely reported.

As the currently used probiotics, prebiotics and synbiotics all lead to the formation of metabiotics that confer most of the gut microbiomes benefit, the most significant portion of a healthy microbiome are the substances produced by the microbiota by digesting the fibre that humans cannot. These exert profound and far-reaching effects on GI system, immune system, general metabolism and inflammation levels. A relationship among all these four types of gut biotics is depicted in Fig. 5.1.

In fact, the emergence of clustering the microbiota based on their functional significance, also known as "phylometabolic core of intestinal microbiota", clearly points to the significance of the metabolites secreted by them towards the host health. The new system classifies the microbiota as butyrate-producing bacteria,

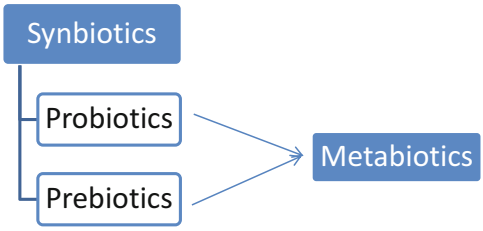

Fig. 5.1 Relationship among probiotics, prebiotics, synbiotics and metabiotics

propionate-producing bacteria, acetate-producing bacteria, hydrogenotrophic bacteria, reductive acetogens, sulphate-reducing bacteria, methanogens, lactate-producing and lactate-utilizing bacteria, bacteria involved in bile acids metabolism, bacteria that metabolize proteins and amino acids, vitamin-producing microorganisms, oxalate-degrading bacteria etc. The concept of metabiotics, therefore, is also able to take care of the issue of microbial dysmetabolism that the probiotics and synbiotics are not capable of (Sitkin et al. 2016).

5.2 Major Components of Metabiotics

Various components of metabiotics, which play a significant role in prevention and treatment of CRC, are illustrated in Fig. 5.2.

5.2.1 Short-Chain Fatty Acids (SCFAs)

SCFAs are the most widely reported component of metabiotics as they not only provide energy for colonocytes but also modulate various metabolic activities. SCFAs are aliphatic carboxylic acids of 1–6 carbon chains including acetate,

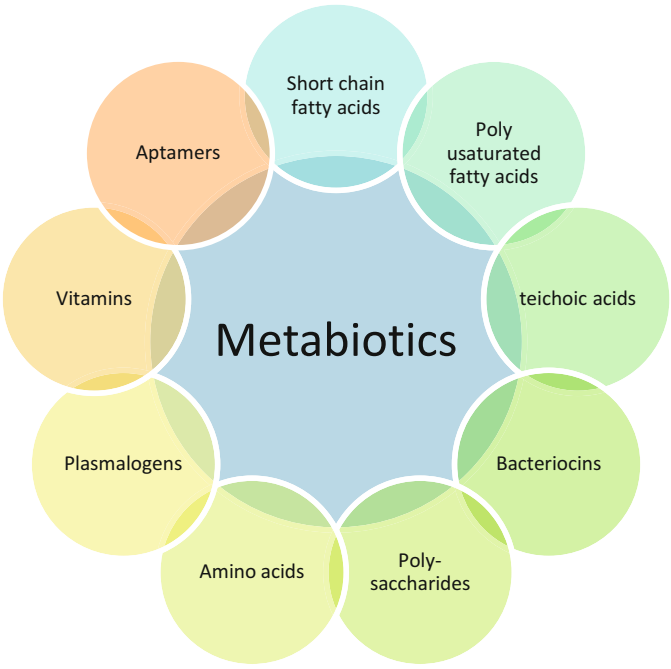

Fig. 5.2 Various components of metabiotics

propionate, butyrate, iso-butyrate, valerate, iso-valerate and hexanoate produced by anaerobic fermentation of dietary fibres in the intestine. Among these, acetate (C2), propionate (C3) and butyrate (C4) are the most abundant ones. The main substrates for SCFAs include resistant starches, various brans like those of oat, wheat etc., cellulose, Guar gum, Xanthan gum and pectin. In the human gut, among the gram-negative bacteria, Bacteroidetes are the most dominant species producing acetate and propionate. However, among the gram-positive bacteria, Firmicutes most predominantly produce butyrate. Acetate, which is produced from acetyl-CoA derived from glycolysis, is the most abundant SCFA in the gut. Acetate molecules are transformed into butyrate by the enzyme butyryl-CoA:acetyl-CoA transferase. Relatively much less is reported about the role of formate in the microbiome equilibrium. It has been reported to influence methanogenesis and is elevated in inflammatory conditions (Morrison and Preston 2016). The main molecular targets involved in the mechanism of action of SCFAs include various G-protein coupled receptors, free fatty acid receptors and hydroxy-carboxylic acid receptors, particularly, GPCR43/FFAR2, GPCR41/ FFAR3, GPCR109A/HCA2, GPCR81/HCA1, HDAC1 and HDAC3. Butyrate is reported to downregulate Placental-specific 8 (PLAC8) expressions and induce apoptosis in PLAC8-overexpressing cells. It is pertinent to note here that PLAC8 cells exert tumorigenic and invasive effects on colorectal cells (Huang et al. 2020).

The main producers of the SCFAs in the gut are listed in Table 5.1.

Gut health and immune function are strongly modulated by the abundance and ratio of absorbable SCFAs. There are a number of pathways through which SCFAs interact with the host. They signal through G-protein-coupled receptors such as GPR41 and GPR43 affecting the critical processes including inflammation, proteins related to tight junctions and regulation of enteroendocrine system. They also maintain an acid pH that supports the growth of certain beneficial bacteria. As SCFAs are absorbed by the host in exchange for bicarbonate, the colonic pH is the result of the production of SCFA and the neutralizing capacity of bicarbonate. Release of peptide YY and of glucagon-like neuropeptide-1 (GLP-1) from enteroendocrine cells is stimulated by propionate, butyrate and acetate SCFAs. These, in turn, modulate lipid metabolism and affect the liver function. Being a dominant energy substrate for colonocytes, butyrate is reported to release approximately 1000 kcal/day (Lazar et al. 2019). Moreover, GLP-2 release and mucus secretion stimulated by butyrate leads to decrease in the permeability of the gut barrier and thus protects against colitis and CRCs. Interplay between diet that acts as substrate for the gut microbiota and host metabolism, resulting in changes in production of SCFA and the microbiota, is reported to have significant effects on host metabolism (Feng et al. 2018). It is well-known that the energy metabolism of CRC cells is different from that of normal cells. An unusual observation that butyrate stimulates the proliferation of normal host cells but induces apoptosis in CRC cells known as the “butyrate paradox” constitutes another mechanism by which SCFAs exert their effect in CRC (Vakhitov et al. 2016).

Certain modification in the expression of certain genes like histone modifications including methylation, phosphorylation, deacetylation and remodelling of chromatin

Table 5.1 Details of production of SCFAs in human gut

Parameter	Acetate	Propionate	Butyrate	References
Producers in gut	*Lactobacillus* spp.	*Phascolarctobacterium succinatutens*	*Faecalibacterium prausnitzii*	Louis and Flint (2009), Shimizu et al. (2018), Morrison and Preston (2016)
	Bifidobacterium spp.	*Roseburia faecis*, *R inulinivorans*	*Clostridium leptum*	
	Akkermansia muciniphila	*Serratia* spp.	*Eubacterium rectale*, *E. ramulus*, *E. hallii*, *E. cylindroides*	
	Bacteroides spp.	*Salmonella enterica*	*Roseburia intestinalis*, *R. faecis*, *R. inulinivorans*	
		Pseudomonas spp.	*Butyrivibrio fibrisolvens*	
	Prevotella spp.	*Bacteroides uniformis*, *B vulgatus*	*Anaerostipes caccae*	
	Ruminococcus spp.	*Prevotella copri*	*Coprococcus catus*, *C. eutactus*, *C. comes*	
	Streptococcus spp.	*Alistipes putredinis*	*Subdoligranulum variabile*	
		Eubacterium hallii	*Anaerotruncus colihominis*	
		Dialister spp.		
		Megasphaera elsdenii		
Pathways	Wood–Ljungdahl pathway	Succinate pathway	Butyryl-CoA:Acetate CoA-transferase pathway	Feng et al. (2018)
	Acetyl-CoA pathway	Acrylate pathway	Phosphotransbutyrylase/butyrate kinase routes	
		Propanediol pathway		

Table 5.2 Pathways of action of SCFAs

S. no.	Pathway of action	References
1.	Decrease colonic pH	den Besten et al. (2013)
2.	Inhibit growth of pathogens	Tan et al. (2014)
3.	Improve integrity and function of colonic epithelial cells	Liu et al. (2014)
4.	Enhance immune function	Nastasi et al. (2015)
5.	Reverse/prevent epigenetic changes	Licciardi et al. (2010)
6.	Host cell proliferation	Vakhitov et al. (2016)
7.	Downregulate (PLAC8) expressions and induce apoptosis in PLAC8-overexpressing cells	Huang et al. (2020)

that occur without any changes in DNA sequences (known as epigenetic changes) may lead to transformation of the normal colonic cells to CRC cells. Such transformations are brought about by certain enzymes like histone deacetylases that silence the tumour suppressor genes. SCFAs act as histone deacetylase inhibitors and are capable of reversing these epigenetic changes (Licciardi et al. 2010). Various pathways by which the SCFAs exert their effect are summarized in Table 5.2.

5.2.2 Polyunsaturated Fatty Acids (PUFAs)

Colonic bacteria have been reported to produce polyunsaturated fatty acids (PUFAs) that contribute towards modulation of angiogenesis, apoptosis and immune response (Bassaganya-Riera et al. 2004). The long-chain acids like γ linoleic acid (GLA), linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, punicic acid and eleostearic acid strongly influence the induction and progression of inflammatory and neoplastic diseases. GLA and its peroxidized derivatives bind to DNA to suppress the anti-apoptotic oncogene expression, resulting in its pro-apoptotic effect exhibited in certain gastrointestinal cancer cell lines (Serini et al. 2009). Interestingly, the pro-apoptotic effect of PUFAs like GLA and arachidonic acid has been found to be significantly more pronounced in case of neoplastic cells than in normal cells (Seegers et al. 1997).

Conjugation of these PUFAs, specifically linoleic and linolenic acid, has been reported to render them safer vis-à-vis their unconjugated counterparts (Bhattacharya et al. 2006). Conjugated linoleic acid (CLA) and conjugated linolenic acid (CLNA) are involved in modulation of PPAR γ (peroxisome proliferator-activated receptors γ)-dependent mechanisms leading to anti-inflammatory and anti-carcinogenic effects. Certain isomers of CLA, particularly the all *trans* t9, t11 isomer, have been reported to be most effective in the induction of apoptosis in colon cancer cell lines.

Interestingly, arachidonic acid (AA), though reported to lead to formation of pro-inflammatory and pro-neoplastic eicosanoids, has been shown to exert pro-apoptotic and anti-neoplastic effects in its unesterified form, when added exogenously. Apoptosis induced by AA in colon cancer cells has been attributed to loss of mitochondrial membrane, activation of capsase-3 and capsase-9 and build-up of ROS (reactive oxygen species), suppressing multiplication of neoplastic cells (Zhang et al. 2015). Eicosapentaenoic acid as well as docosahexaenoic acid, the two most studied *n*-3 PUFAs, have also demonstrated pro-apoptotic action by downregulating the effects of cyclooxygenase-2 (COX-2), dual-phosphatase, mitogen-activated protein kinase-1 (MKP-1), β-catenin and surviving, which are reported to be involved in early stages of sporadic neogenesis (Jakobsen et al. 2008).

5.2.3 Mitogen-Activated Protein Kinase

5.2.3.1 Bacteriocins

Bacteriocins are cationic anti-microbial peptides produced by archaea and bacteria that inhibit the growth of other microorganisms at nanomolar concentrations while being harmless to the cells producing them. Various classes of bacteria produce different varieties of bacteriocins that differ in their physico-chemical properties as well as the niche areas of their action. The bacteriocins effective in colon cancer include colicins, microcins, pediocins, nisin etc. Cancer-selective cell lysis by bacteriosins is attributed to the presence of negative charge on their cell membrane and their higher membrane fluidity as compared to that of the normal cells. Bacteriocins because of their cationic nature and membrane destabilizing properties are able to preferentially bind with the cancer cells. Apart from this, the presence of much larger number of microvilli on the cancer cell surface as compared to their normal counterparts leads to higher binding of bacteriocins simply due to the availability of larger surface area (Hoskin and Ramamoorthy 2008). The anticarcinogenic effect of bacteriocins has been attributed to mutation of suppressor genes, cell cycle alterations and generation of pores in plasma membrane, eventually leading to necrosis and apoptosis. The details of bacteriocins reported to be effective in colon cancer are given in Table 5.3.

5.2.3.2 Polysaccharides

Gut microbes synthesize a wide variety of carbohydrates that may remain inside their cytoplasm, become structural, be a part of their microbial envelope or may be

Table 5.3 Bacteriocins reported to be effective in colon cancer

S. no.	Bacteriocin	Source	References
1.	Colicins	*E. coli*	Lancaster et al. (2007)
2.	Microcins	*E. coli*, *Klebsiella pneumoniae*	de Lorenzo (1984)
3.	Pediocins	*Pediococcus acidilactici*	Papagianni and Anastasiadou (2009)
4.	Nisin	*Lactococcus lactis*	Liu and Hansen (1990)

secreted out of the cells as exopolysaccharides (EPS). EPSs from lactic acid bacteria (LAB) have been the most widely studied for their pharmacological effect, including that against colon carcinoma. A number of species of LAB like *Lactobacillus acidophilus*, *L. casei*, *L. rhamnosus* and *L. plantarum* have been reported to exhibit tumoricidal activity by anti-oxidant, pro-apoptotic and immunomodulatory actions. Notable upregulation in caspase activity was observed in the human colon cancer cell line HT-29 by various strains of *L. casei* and *L. rhamnosus* (Di et al. 2018). In a study on colon cancer cell lines, the EPSs from *L. acidophilus* were found to downregulate the expression of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor-1α while upregulating the expression of tissue inhibitor of metalloproteinases-3, hypoxia-inducible factor-2α, and hemeoxygenase-1 and plasminogen activator inhibitor-1 (PAI-1) (Deepak et al. 2016). A penta saccharide extracted from *L. acidophilus* was reported to operate through both apoptotic and NF-κB inflammatory pathways in inhibition of progression of colon cancer. It also exhibited a potential to upregulate the expression of IKbα, P53 and TGF genes (El-Deeb et al. 2018). In an in vivo study, peptidoglycans from LAB species were reported to decrease the growth of CT26 colon cancer cells in Bagg Albino (BALB) mice by their pro-apoptotic activity. The effect was found to be dose dependent in nature (Sun et al. 2005). In a similar study, peptidoglycan from *L. paracasei* has been reported to exert inhibitory effects on colon cancer (Wang and Wang 2010).

Immunomodulatory effects of EPSs from *L. johnsonii* are reported via binding to pattern recognition receptors (PRRs) expressed on immune cells (Górska-Frączek et al. 2013). In another study with the EPSs from *L. kefiranofaciens*, regulation of protective immunity, maintenance of intestinal homeostasis, enhancement of IgA and cytokine production were reported (Vinderola et al. 2006).

5.2.3.3 Amino Acids and Peptides

A number of amino acids produced by the gut microbiota and probiotics are involved in the intestinal microbiota and host crosstalk. Probiotics like *L. paracasei* and *L. rhamnosus* are known to produce glutamine, alanine, glycine, lysine and other branched amino acids. Glutamine is the most abundant free amino acid in the body whose role in development and propagation of colorectal cancer is very crucial. However, the reports regarding the role of glutamine are quite contradictory. Anti-oxidant properties of glutamine are instrumental in reducing the complications of colorectal cancer treatment by reducing mucositis, diarrhoea and neuropathy induced by chemotherapy (Decker-Baumann et al. 1999). Some clinical studies, however, suggest that intake of glutamine acts as an important contributor towards tumour growth and may lead to increase in tumour cell turnover in GIT that it may lead to more growth of these cells (Goldin et al. 1996). Certain novel anti-cancer peptides comprising three amino acids, i.e. arginine, lysine and a non-polar amino acid such as alanine or valine have been identified that inhibit proto-oncogene tyrosine-protein kinase, Src. It is pertinent to add here that Src is instrumental in cancer progression of many tumours including colorectal cancer (Agrez et al. 2012).

The contradictory response of tumorigenesis of glutamine extends to other amino acids as well. Elimination of amino acids like glutamine, glycine, proline and serine

from diet has been reported to antagonize tumour development (Maddocks et al. 2013). In contrast, certain formulations containing essential amino acids were found to induce apoptosis in HeLa, HCT116, MCF7, HepG2 and CaCo2 cell lines. This study showed that the availability of essential amino acids in excess of non-essential amino acids creates a paraphysiological condition that is healthy for normal cells but causes fragility in cancer cells (Bonfili et al. 2017).

Ferrichrome, a cyclic hexa-peptide that forms a complex with iron atoms, has been isolated from the cell cultures of *L. casei*. It has a strong tumour-suppressive effect on colon cancer cells, even greater than that of commonly used anti-cancer drugs like cisplatin and 5-fluorouracil. Moreover, its cytotoxic effect on non-cancerous intestinal cells is less than that of the chemotherapeutic agents making it safer. Its tumoricidal effect is attributed to the induction of apoptosis mediated by the inhibition of the c-Jun N-terminal kinase (JNK) signalling pathway (Konishi et al. 2016).

A peptide secreted by *L. lacti*, KiSSpeptin, was found to inhibit human colon carcinoma HT-29 cells proliferation and metastasis by enhancing apoptosis and downregulating the expression of metallopeptidases (Zhang et al. 2016).

5.2.3.4 Plasmalogens

Plasmalogens are phospholipid molecules with unique 1-O-alk-10-enyl 2-acyl glycerol phospholipid and glycolipid composition, which are found in many strictly anaerobic bacteria including most species of Clostridium (Goldfine 2010). Plasmalogens are located in the cell membrane and organelles, being the major constituents of membrane lipids. Having very strong intermolecular hydrogen bonding between the individual molecules, plasmalogens have lower lamellar gel to liquid-crystalline temperature as compared to their alkyl and diacyl counterparts. They are reported to decrease tumour cell invasion and also inhibit the process of metastasis (van Blitterswijk and Verheij 2013). Due to their structural similarity with the membrane lipids, they interfere with lipid homeostasis, leading to apoptosis due to alteration in lipid linked signalling. Moreover, plasmalogens are well reported for their anti-oxidant effect. Their anti-oxidant effect is attributed to their susceptibility to ROS-mediated cleavage due to high electron density of the vinyl ether bond at the sn-1 position, accessibility of the vinyl ether linkage to ROS due to their location the hydrophilic domain of membrane and slow propagation of the plasmalogen hemiacetal hydroperoxy radicals. High levels of plasmalogen in the mutant cells are reported to protect them from chemical hypoxia (Zoeller et al. 1999). Plasmalogens also act as lipid mediators for cell signalling. Docosahexaenoic acid released by the action of lysoplasmalogens acts as a precursor for resolvins and protectins, which, in turn, remove chemokines and regulate leukocyte infiltration leading to termination of acute inflammation in tissues (Wallner and Schmitz 2011). Motivated by their success in combating colon cancer, a number of synthetic plasmalogens have been prepared and are being tried for treatment of cancer (Messias et al. 2018). The role of plasmogen in colon cancer is shown in Fig. 5.3.

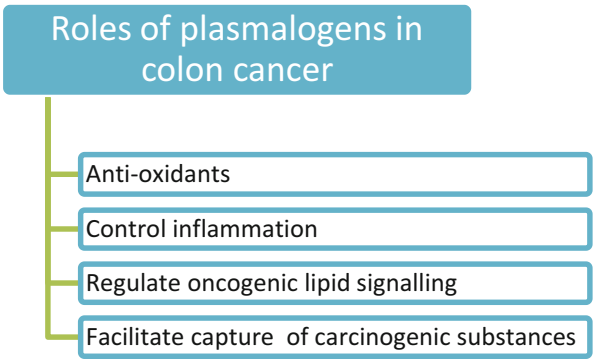

Fig. 5.3 Role of plasmalogens in colon cancer

5.2.3.5 Vitamins

Probiotics are widely reported to produce vitamins, especially vitamins of group B and folate. Folate deficiency is reported to increase the risk of colorectal cancer (LeBlanc et al. 2011). Vitamin B6 has been reported to reduce the risks of certain gastro-intestinal tumours (Mocellin et al. 2017).

Vitamin D has been reported to be produced by certain LAB strains (Jones et al. 2013). A number of epidemiological studies have shown an association of vitamin D deficiency with occurrence of CRC. Calcitriol (1α,25-dihydroxyvitamin D3), a metabolite of vitamin D, inhibits the proliferation of colon cancer cell lines. In cell line studies, calcitriol has been shown to modulate gene expression and inhibit the pro-tumoural properties of colon cancer-associated fibroblasts. Regulation of vitamin D receptors on various classes of immune cells also contributes towards the suppression of colon cancer by vitamin D. Vitamin D promotes tumoricidal activity of macrophages and improves the efficacy of antibody-dependent cellular cytotoxicity.

5.2.3.6 Aptamers

Aptamers, the small, single-stranded DNA or RNA molecules ranging between 25 and 100 nucleotides constitute a significant component of metabiotics.

In one of the initial studies, a multivalent RNA aptamer, prostate-specific membrane antigen (PSMA-4-1BB) was found to inhibit the growth of colorectal cancer (Santulli-Marotto et al. 2003). Another RNA aptamer has been reported to target angiogenesis through the peroxisome proliferator-activated receptor δ (PPAR-δ), leading to decrease in the neogenicity of colorectal cancer cells (Kwak et al. 2009). Certain aptamers like YJ1 aptamer have been demonstrated to not only suppress the colorectal cancer but also to prevent its hepatic metastasis. This is attributed to its activity against carcinoembryonic antigen (CEA), which is known to lead to cell adhesion and cancer cell migration to the liver leading to hepatic metastasis (Lee et al. 2012).

Some DNA aptamers, like minimal primer7 (MP7), are reported to block pathways programmed by cell death proteins, PD-1/PD-L1, and inhibit the growth of colorectal cancer (Schrand et al. 2014).

5.3 Edge Over Probiotics

The role of probiotics in colorectal cancer prevention and treatment has been reported extensively in a number of clinical and molecular studies. The mechanisms that have been indicated for the action of probiotics against colorectal cancer include alteration of the intestinal microflora; inactivation of carcinogenic compounds; competition with putrefactive and pathogenic microbiota; improvement of the host's immune response; anti-proliferative effects via regulation of apoptosis and cell differentiation; fermentation of undigested food and inhibition of tyrosine kinase signalling pathways. The probiotic strains that have been found to be useful include *Bifidobacterium adolescentis*, *B. lactis*, *Enterococcus faecium*, *Lactobacillus rhamnosus*, *L. casei*, *L. paracasei*, *L. salivarius*, *Bacillus polyfermenticus*, and *Pediococcus pentosaceus*.

Despite a long history of safe use, introduction of live bacteria in the body is speculated to be associated with the risk of systemic infections, especially in case of genetically predisposed and immunocompromised hosts. A number of cases are reported wherein the use of probiotics has led to development of bacteraemia, fungaemia and endocarditis in both immunocompromised and non-compromised patients (Bassetti et al. 1998). Another concern raised by the 2002 report jointly released by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations (http://www.fao.org/3/a-y5861e.pdf) is that of deleterious metabolic activities.

In a double-blind randomized controlled trial of a combination of six probiotics, i.e. *L. acidophilus*, *L. casei*, *L. salivarius*, *L. lactis*, *B. bifidum and B. lactis*, in 296 patients with severe pancreatitis, a higher mortality was reported in patient group using probiotics as compared to the placebo group. Administration of probiotic was speculated to lead to increase in oxygen demand in addition to already reduced blood flow leading to intestinal ischaemia (Besselink et al. 2008). Some reports of acidosis due to the production of D-lactate by the probiotics are cited in patients with short bowel syndrome and are also available in literature (Ku et al. 2006; Reddy et al. 2013). In fact, the need of probiotic use to strengthen the clinical treatments in various diseases seems to be most needed in case of immunocompromised patients, but unfortunately this group of patients stands out as the one which is deprived of their therapeutic advantages due to perceived risks. There also exists some scepticism regarding the concentration of bioactives produced by probiotics at the target organs (Shenderov 2013).

Another controversial aspect of probiotics that remains hitherto neglected involves the capability of these microbes to transform certain drugs leading to change in their pharmacokinetics. In this case also, the irony of the situation is that most of the diseases where the probiotics are used to strengthen the pharmacotherapy already require the administration of a number of drugs. Administration of multi-strain probiotics or genetically modified microorganisms further increases the complexity of the situation. Unfortunately, there are no substantial scientific reports, either in vitro, ex vivo or in vivo exist in the literature regarding the effect of probiotics with drug pharmacokinetics (Clayton et al. 2009).

Table 5.4 Commercially available metabiotic formulations

S. no.	Product	Manufactured by	Recommended for	References
1.	Hylak Forte	Ratiopharm/ Merckle, Germany	Constipation, gas, bloating, nausea, headache, fatigue	Gasilina and Belmer (2015)
2.	Zakofalk	Dr. Falk Germany	Inflammatory intestinal diseases	Roda et al. (2007)
3.	Bactistatin Gut®	Kraft Group of companies	Immunomodulator	Vorobeĭchikov et al. (2008)
4.	Aktoflor C	Solopharm	Pneumonia	Spencer and Chesson (1994)
5.	Lacteol Forte	Cipla, Carnot etc.	IBS	Halpern et al. (1996)
6.	Kodivak	Irkutsk State University	Acute respiratory disease	
7.	Acilact	Metapharm	Gingivitis	Lykova (2001)
8.	Nagipol	Bitra	Restores metabolism and digestive disorders	Dotsenko et al. (2004)

As evident, most of the limitations of probiotics accrue from their live nature. Metabiotics have been developed, therefore, as more specific and measurable alternative with less scepticism in the scientific society regarding their unanticipated effects. A number of metabiotics have been developed and are already in clinical use. Interestingly, they have been recently reported to have been added to fortify the special rations prepared for the Russian armed forces posted in arctic region (Artyukhova et al. 2019). Information regarding commercially available metabiotic formulations is reported in Table 5.4.

5.4 Conclusion

The efficacy of metabiotics in the treatment of CRC is now well established. As more metabiotics are engineered and more clinical data are created, metabiotics are expected to become a supportive therapy, if not the first line treatment in colon cancer. Carefully conducted double-blind, placebo-controlled trials individually document the efficacy of each specific component and will render the metabiotic therapy more rational and effective. Use of clinically proven metabiotics as supplements could lead to paradigm shift from treatment to prevention aspects of CRC. There is a need to conduct extensive clinical trials, especially to find out the metabiotic components that prevent metastasis and generate evidence thereof. Looking at the global development and commercialization of the metabiotic products across national borders, implementation of internationally harmonized regulations for each aspect of this evolving class of bioactives is the need of the hour. Identification of the probiotic components having optimum activity against

various CRC cell lines from different strains of bacteria, their isolation/synthesis and their cost effective as well as safe formulation are the other areas that need focus.

Considering the variation in types of dysbiosis and the individuality of gut microbiota structure, the focus should be shifted to personalized metabiotic therapies in CRC, before using them routinely. In fact, looking at the various types of dysbiosis and the individuality of gut microbiota structure, the future of CRC treatment seems to lie in the integrated "-otics" platforms. The conventional treatment could be initially supported aggressively by the co-administration of synbiotics and metabiotics, subsequently stabilizing on synbiotics, which, in turn, may be supported by long-term dietary interventions to provide sustained therapeutic payloads of metabiotics.

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Possibility of Probiotic in Colorectal Cancer: A Specific Countenance to Research

6

Mohammad Yasir, Ruchi Khare, Pushpendra Singh, Sohni Singh, and Rahul Shrivastava

Abstract

Colorectal cancer (CRC) is one of the most deadly groups of diseases. Apart from genetic mutations, other aspects such as environmental factors, unhealthy lifestyle and poor diet cause distress in gastrointestinal environment by changing physio-biochemical properties of luminal content which provides favourable environment for carcinogenesis. Administration of probiotics is helpful in prevention of carcinogenesis along with maintenance of microbial balance. They modulate patient's gut during systemic cancer therapy and suppress oncogenic properties of the tumour cells by manipulating enzymes involved in mucosal immune response, inflammatory pathways, cell differentiation and proliferations. This chapter highlights huge scientific importance of probiotics as bio-therapeutics in cure and early prevention from CRC, with recent findings on anticancer effects, in vitro cell line and in vivo animal studies, safety and regulatory issues, challenges, precautions and future directions.

Keywords

Colorectal cancer · Dysbiosis · Microbiome · Probiotics · Polyps

M. Yasir (✉) · R. Khare · P. Singh · R. Shrivastava
Department of Biological Science and Engineering, Maulana Azad National Institute of Technology, Bhopal, Madhya Pradesh, India
e-mail: shrivastavarm@manit.ac.in

S. Singh
School of Computing and Information Systems, University of Melbourne, Parkville, VIC, Australia

P. K. Deol (ed.), *Probiotic Research in Therapeutics*,
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6.1 Introduction

Cancer is a group of diseases marked by uncontrolled growth and invasion of cells in the blood stream, i.e. metastasis. According to the site of origination, different cancers can be characterized as head and neck cancer, breast cancer, prostate cancer, lung cancer and many others. In which colorectal cancer (CRC) is a type of common and curable cancer, but resistance to chemotherapy has emerged as a new challenge. Lilly and Stillwell coined the term "probiotics" as "viable microorganisms" that stimulate the growth of other microorganisms and confer health benefits on host when administrated in adequate amount (Markowiak and Śliżewska 2017). As microorganisms have symbiotic relationship with human digestive system, they play a key role in maintaining gut environment. The microorganisms acting as probiotics have various functions such as modulation of normal immunological response of intestine, inactivation of carcinogenic compounds, providing various nutrients, activation of enzymes, etc. Mostly studies have reported the potential of lactic acid bacteria (LAB) as probiotics showing anticancer activity and these LAB are extensively used in dairy products and fermentation products (Uccello et al. 2012).

Colorectal cancer originates from colon or rectum epithelium cells (polyps) and advances as adenomatous polyp to invasive cancer (Gryfe et al. 1997). It is the third most common cancer in the world and the fourth most common cause of morbidity and mortality (Haggar and Boushey 2009). It is not a hereditary syndrome, mostly environmental risk factors are said to be the cause which also include dietary habits, lifestyle and social routines. High fat diet favours development of bacterial flora capable of degrading bile salts to potentially carcinogenic N-nitroso compounds (Larsson and Wolk 2006; Janout and Kollarova 2001; Santarelli et al. 2008).

6.2 Classification of Colorectal Cancer

Colorectal cancer can be characterized based on genetics as:

6.2.1 Familial Adenomatous Polyposis (FAP)

FAP is an inherited autosomal dominant disease caused by germline mutation in adenomatous polyposis coli (*APC*) gene (Bisgaard et al. 1994). In normal condition, β-catenin gene transcribes β-catenin protein which acts as transcription factor, responsible for increase in proliferation of cells. This β-catenin protein also interacts with E-cadherin, responsible for cell adhesion. The proliferation and adhesion by β-catenin are regulated by APC protein, in which APC protein acts as a negative regulator in the pathway. *APC* gene destroys β-catenin protein in Wnt signalling pathway for controlled proliferation of cells.

FAP mutations (e.g. insertion, deletion, missense mutation) lead to the production of truncated APC protein, which is also known as DP 2.5 (deleted in polyposis 2.5).

This event leads to the accumulation of β-catenin protein resulting in uncontrolled growth of epithelium cells (polyps). These polyps progress from benign condition to metastasis and later result in colorectal cancer. Less aggressive variant polyps found in proximal colon and rectum are characterized by fewer (less than 100) adenomatous polyps known as attenuated FAP (AFAP), they appear at later age and have lower cancer risk (Talseth-Palmer 2017).

6.2.2 MUTYH-Associated Polyposis (MAP)

This adenomatous polyposis has bi-allelic germline mutation in gene *MUTYH* by base-excision repair (*BER*) mechanism. Patients of this disease are not diagnosed with FAP or AFAP because it is not associated with APC gene mutation, although after diagnosis the disease management is similar to FAP. The common site of origination is upper intestine and it has 80% chances to propagate as CRC.

6.2.3 Serrated Polyposis Syndrome (SPS)

SPS is a rare syndrome, also known as hyperplastic polyposis syndrome, identified by the presence of multiple serrated polyps in colon. Serrated polyposis syndrome is characterized by:

1. Presence of at least 5 serrated polyps in which size of two should be >10 mm.
2. An individual with first-degree relative with SPS also having serrated polyps proximal to sigmoid.
3. Any size of polyps with >20 in no. and it is distributed throughout the colon (Rex et al. 2012).

6.2.4 Hereditary Non-polyposis Colorectal Cancer (HNPCC)

It is also known as lynch syndrome, most commonly an inherited colon cancer syndrome. It is autosomal dominant genetic condition characterized by microsatellite instability (MSI) as a hallmark. Inactivation of remaining normal allele and germline mutation in *MMR* gene leads to defects in DNA which further cause microsatellite instability (MSI) (Fearon 2011).

6.2.5 Sporadic Colon Cancer

The main reason behind cancer is amassing of multiple genetic alterations in the epithelium cells of colon. This amassing of mutation causes selective growth of epithelium cells progressing as adenoma carcinoma leading to sporadic cancer.

6.3 Effect of Probiotics on Colorectal Cancer Pathways

An imbalance of intestinal microflora links diet and CRC (Uccello et al. 2012). *Lactobacillus GG*, a probiotic showed up-regulation of 334 genes and down-regulation of 92 genes in small bowel mucosa cells. In this study, gene expression of proteins involved in immune response and inflammation via MAPK pathway (TGF and TNF family members, cytokines, nitric oxide synthase 1, defensin α1), apoptosis, cell growth and cell differentiation (cyclins and caspases, oncogenes), cell–cell signalling (ICAMs and integrins), cell adhesion (cadherins), signal transcription and transduction was analysed (Di Caro et al. 2005). Action of various probiotics on three different pathways responsible for colorectal cancer has been explained, i.e. CIN (chromosomal instability) pathway, MSI (microsatellite instability) pathway and CIMP (CpG Island methylation) pathways in Fig. 6.1.

6.4 In-Vitro Studies on Probiotics

In-vitro studies dealing with effect of probiotics on colorectal cancer are mainly focussed on different human cell lines and bacteria/bacterial cell extracts showing anticancer, anti-inflammatory properties, antibiotic resistance and the pathways

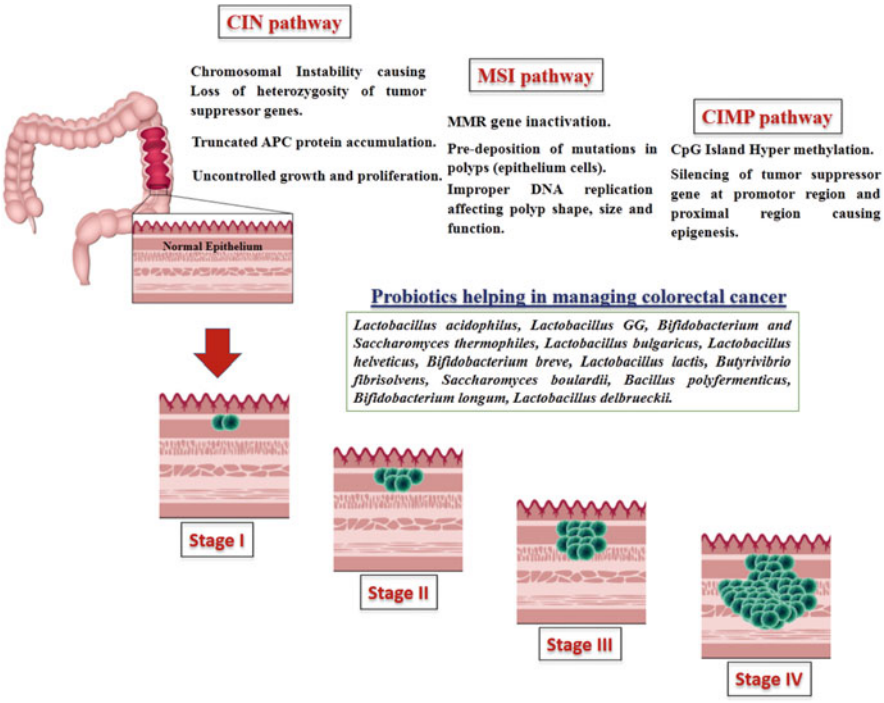

Fig. 6.1 Action of various probiotics on three different pathways responsible for colorectal cancer

related to them. The group of LAB microorganisms include different genera such as *Bifidobacterium*, *Pediococcus*, *Enterococcus, Lactococcus*, *Streptococcus* and *Lactobacillus* in which lactobacillus has shown maximum technical characteristics of good probiotics. Although probiotic cultures are developed in different flavoured products for better consumption, dairy products like fermented milk have also been used to study the role of probiotics as biotherapeutics. Culture manufacturing technology for producing fermented milk contains *Bifidobacterium* strains as ABT cultures (ABT standing for *L. acidophilus, Bifidobacterium* and *S. thermophilus*) (Tamime et al. 1995).

Milk slots were fermented by each of the bacterial populations of *Bifidobacterium, L. acidophilus* or in combination of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. In each of the fermented milk HT-29 cells were inoculated and kept under observation. Bacterial strains of *Lactobacillus helveticus, Streptococcus thermophilus* and *Lactobacillus bulgaricus* caused drop in the growth rate of HT-29 cells at a significant and variable level, this also caused reduction in cell count at steady-state by 10–50%. A blend of two probiotics *Bifidobacterium breve* R0070 + *Lactobacillus lactis* R1058 + oligoalternan subdued rapid increase in number of HT-29 cells without cytotoxic effect (Grimoud et al. 2010). In another in vitro human probiotic study *Propioni bacterium* spp. destroyed CRC cells through apoptosis via its three metabolites, the short-chain fatty acids (SCFA), acetate and propionate (Jan et al. 2002; Lan et al. 2007).

Research involving use of cell extracts of *Lactobacillus acidophilus* LA102 and *Lactobacillus casei* LC232 against colorectal cancer (CRC) cell lines, Caco-2 and HRT-18, demonstrated that isolates exhibit proliferation inhibition of 37% and 68.5% in case of *Lactobacillus acidophilus*, and 48% and 45.7% in case of *Lactobacillus casei* against Caco-2 and HRT-18. The IC_{50} values were reported to be 1.6 and 2.5 μg/ml of LA102, and 15.4 and 6.2 μg/ml of LC232 against Caco-2 and HRT-18. These interpretations raise the visions of using probiotic for possible cancer prevention and even treatment (Awaisheh et al. 2016).

Many recent studies have shown that table olives are a source of probiotic bacteria, mainly those fermented by using traditional procedures. Several studies have been done in recent years which demonstrate that LAB species obtained from table olive cultivars have probiotic features. It is worth mentioning that the property of probiotic has shown promising results in treating CRC and a recent study has identified several potential probiotic candidates from different sources which could help fight cancer. In one of the studies performed, nearly 31 *Lactobacillus pentosus* strains extracted from Alorena green table olives were tested for probiotic properties (Guantario et al. 2018). The results suggest that LAB is a potential candidate to manufacture fermented table olives. In another recent study performed on two strains of lactic acid bacteria isolated from table olives were screened to study the probiotic potential (Blana et al. 2014). Dynamics of population of the both the strains was studied for the period of 114 days. Both the strains of probiotics colonized the surface of olive. Biochemical profiling studies during the fermentation process indicated lactic acid fermentation process of green olives.

Cancerous cells were treated with *Lactobacillus casei* ATCC 393 and it shows decrease in cell viability. It was also observed that live *Lactobacillus casei* induced apoptotic cell death. The results provided evidence that *Lactobacillus casei* ATCC 393 has inhibitory, pro-apoptotic and anti-tumour effects on in-vitro models (Tiptiri-Kourpeti et al. 2016).

Enterococcus lactis is a potential probiotic found in the human gut and was tested for its anticancer properties. *Enterococcus lactis* when treated with a variety of cancerous cells such as HeLa, MCF, MCF-7 and Caco-2, acts as a growth inhibitor to model cancer cells. Study shows that HeLa cells under the effect of *Enterococcus lactis* underwent apoptosis—a main cytotoxic effect. In this study, the test was performed to study the effect of metabolites secreted by *Enterococcus lactis* IW5 on different selected cancer cell cultures. The assay results indicated that metabolites are responsible for inhibition of cancer cell growth (Nami et al. 2015).

6.5 In-Vivo Studies on Probiotics

Strain of butyrate producing rumen bacterium *Butyrivibrio fibrisolvens* MDT-1 was studied on mice model, with lowering of luminal pH. It was observed that mice produced high amounts of butyrate because of this faecal microbe. It is suggested that possible growth of probiotic in gut and its association with a reduced cryptic foci suppress the risk of CRC (Ohkawara et al. 2005). Use of probiotic in human studies to prevent CRC using particular human GI disorders like inflammatory bowel diseases are prevented by using *Saccharomyces boulardii* (Sb) as a safety probiotic agent (Guslandi et al. 2000). Sb modulated many host signalling pathways such as MAPK signalling pathway that are up-regulated/down-regulated due to intestinal mucosal inflammatory response. MAPK signalling pathways are intracellular pathways responsible for cell proliferation and regulation. Like many growth-factor receptors, epidermal growth factor receptor (EGFR) family consists of four members: ErbB1/EGFR/HER1, ErbB2/HER2/Neu, ErbB3/HER-3 and ErbB4/HER-4 (Hynes and MacDonald 2009). These receptors play a crucial role in cancer development as these receptors perform intracellular cascade for production of proteins such as CDKs and cyclins, responsible for cell cycle regulation. In animal model, ApcMin mice were used for intestinal tumour study to examine quantitative and mechanistic effects of *Saccharomyces boulardii* (Sb) as probiotic. The probiotic Sb not only suppressed tumour formation and EGFR mediated proliferation, but it also promoted apoptosis of cells (Moser et al. 1995). Probiotic *Bacillus polyfermenticus* prevents in-vitro and in-vivo cell growth, as it showed anticancer effect through ErbB2 and ErbB3 receptors and down-regulation of signalling molecules E2F-1 and cyclin D1 which are responsible for tumour suppression and progression of cell cycle from G1 phase, respectively (Ma et al. 2010). Effect of dietary administration of probiotics on rats was studied. Lyophilized cultures of *Bifidobacterium longum* resulted in suppression of new cancers at specific site of colon, occurrence of different type of tumourigenesis, number of tumours per animal, and also tumour volume (Singh et al. 1997). This suggested effect of

probiotics possesses CRC-protective properties by modifying differentiation process of tumour cells.

In-vivo studies in rat-azoxymethane model demonstrated that synbiotic combination of *Bifidobacterium lactis* and resistant starch significantly protects against the development of colorectal cancer (Le Leu et al. 2010). Mice treated with a probiotic mix composed of seven different strains of *lactobacilli, bifidobacteria*, and *streptococcus* suppress colon carcinogenesis by modulation of mucosal CD4+ T polarization and changes gene expression in azoxymethane-induced colorectal cancer (Bassaganya-Riera et al. 2012; Górska et al. 2019).

In another study modulation of host immune response was observed when C57BL/6 mice were treated orally with *Lactobacillus casei* BL23 in drinking water for up to 10 weeks in 1,2-dimethylhydrazine (DMH) induced disease model (Molska and Reguła 2019).

During the period of 2015 to 2018 many strains of probiotics were tested on CRC patients to study their effectiveness on treating colorectal cancer. Patients treated with different combinations of probiotic strains showed decrease in the risk of postoperative complications. For example, in one of the recent studies 140 colorectal cancer patients when treated with *L. acidophilus, L. lactis, B. bifidum* and *B. Longum* strains of probiotics at 30 billion cfu per sachet at a dose of 2 sachets daily for 4 weeks showed reduction in inflammatory biomarkers and side effect of chemotherapy (Golkhalkhali et al. 2018).

6.6 Efficacy and Safety Concerns of Probiotics in CRC

Many in-vitro and in-vivo studies have explored pathways of microbiome performing cancer-preventative mechanisms that have shown intraluminal and systemic effects on development of tumours and precancerous lesions. But, there is restricted human data in relation to specific manipulation of microbiome assessing colorectal cancer risk.

The treatment with probiotics is prescribed to people suffering from non-cancerous diseases (Redman et al. 2014). But, people suffering from cancer have limited or prohibited use of probiotics as they are mostly immune-compromised and suffered from daily health-care issues like inflammation, infections etc. Colorectal cancer is more concerned with dietary consumption, so administration of any probiotic to patients with this type of cancer requires great attention and pre-clinical studies. But if probiotics are properly administered in adequate amount of dose, they showed positive rebalancing impacts by strengthening of immune system; modulating the GI tract microbiota to fight against infections, inflammations; deactivating the oncogene, excess secretion of cytokines, tumour necrosis factors, tumour growth factors and activating transcription of tumour suppressor genes and cell junction proteins (Wan et al. 2014; de Moreno de Leblanc and Perdigon 2004; Karczewski et al. 2010; Galdeano and Perdigon 2006; Vinderola et al. 2006; Madsen 2012). After assessment of quantitative and qualitative profile of intestinal microbiota consistent uptake of probiotics showed improvement by

sinking initiation of chronic inflammation and production of carcinogenic compounds during intestinal dysbiosis (Liu et al. 2011; Hatakka et al. 2008).

6.7 Regulatory Issues of Using Probiotics in CRC

Regulation of identification and potency of commercial probiotic contents, efficacy and technological function must be evaluated. The use of probiotic requires careful assessment before administering them to immunosuppressed cancer patients. For regulation and management of probiotics production, manufacturing and safety, FDA has appointed an authority on 24 August 2007 under the rules of Good Manufacturing Practices (GMP). In this guideline, the criteria related to identification, characterization, validation, shelf life and misleading contents are given (Indian Council of Medical Research Task Force et al. 2011). The strains of probiotics can be different in in-vivo models. Although, data related to human study is rare. The most common source of probiotics, yoghurt is said to be the best source of these supplements. During culture, both starter cultures (*Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*) and added bacteria for health effects (e.g., strains of other species of *Lactobacillus or Bifidobacterium*) may be present (Sanders 2008). Identification of these strains is beneficial to predict in-vivo function, their documentation of expression and the range of targets for in-vivo functions such as oral, stomach, respiratory, intestinal, vaginal, etc. To examine in-vivo efficacy, other than strains, studies related to their production, coating, and preservation technology, metabolic state of probiotic are yet to be done. To increase the use of probiotics, these areas of research are still untouched.

6.8 Challenges in Probiotics Consumption

With increase in awareness of personal health care amongst the people, other than good food they also need functional food which can prevent illness. For this demand and supply chain in food product market, there is need to study viability and stability of probiotic at industrial level. The product should have specific strains of *Microbacterium* and specific viable cell count within the shelf life of product. The manufactured formulation of probiotics should have the ability to sustain in GI tract environment without causing any adverse reaction to host system. For non-dairy probiotic products, the biggest challenge is to maintain the optimum environment for cell survival such as water activity, oxygen tension, fluctuating temperatures and many more. The products used in baby care and confectionaries are at higher stakes as probiotics mixed in the culture bed should not multiply or change their expression, which can lead to change in texture, role and properties of the products (Saarela et al. 2000). So there are many challenges related to production, manufacturing, formulation and stability which need authentic data generation to exploit use of probiotics in daily life and precautions to be applied.

6.9 Precautions for Using Probiotics

With accelerated research in the field of biotherapeutics, there is need to fix the guidelines regarding oral administration of probiotics to colorectal cancer patients. As colorectal cancer is directly associated with diet and GI tract environment, the adverse effects of probiotics may cause many disorders both inside and outside of GI tract. Several studies in this book chapter have suggested different species of probiotics along with their starter cultures. But, inadequate data on human consumption limits the understanding of the effects of specific probiotics and their predicted biological and physiochemical activity. Probiotics administration should be done under clinical guidance to avoid any kind of infection in patients. Similar to patch test for allergies there is need to set proper tests to check the susceptibility of host for different microorganisms. These precautions showed significance in getting response of probiotics in cancer patients undergone chemical and radiotherapy or at early stage of colorectal cancer.

6.10 Future Directions on Probiotic Research

In spite of insufficient data regarding production, manufacturing, industrial standards and clinical health care, use of probiotics carries great potential in down staging colorectal cancer. For identification and characterization of probiotics used in colorectal cancer, various wet lab research are going on. With the advent of gene technology, there is need to exploit the genetic data present in many gene banks (Parvez et al. 2006). By using bioinformatics tools such as protein–protein docking, quantitative structure activity relationship (QSAR) we can predict the functions of proteins inside the GI tract. Using these tools we can also suggest drugs which can make the probiotics viable, stable and more effective for patients suffering from colorectal cancer.

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Probiotics in Lung Cancer: An Emerging Field of Multifarious Potential and Opportunities

7

Mallesh Kurakula and Koteswara Rao G. S. N.

Abstract

Lung cancer is one of the major causes of mortality and morbidity worldwide adding a significant burden on healthcare cost. Apart from conventional and chemotherapy strategies, use of probiotics as an adjunct therapy for prevention or treatment of tumours was a game changer with scientific proofs from diverse research groups. Probiotics are the specific bacterial or fungal strains—live or dead, along with their metabolites when consumed at certain concentrations indicated proven health benefits. Probiotics are being recognized for their repurposing advantages having immunomodulatory responses and reported as alternative for cancer biotherapeutics. In this chapter, the proposed mechanisms of probiotics' use in lung cancer therapy during proliferation, metastasis, and immunomodulation are discussed. A spolight on the elucidation of probiotics as potential candidates in the management of pulmonary tumour, highlighting relevant in vitro (cell line studies) and in vivo (animal and human trials) studies. New emerging trends using bioengineering recombinant approach of probiotic bacteria against respiratory cancer, their limitations, and future prospectus are outlined in the current chapter.

Keywords

Lung cancer · Pulmonary · Probiotics · Prebiotics · Bacterial · Fungal strains · Repurpose · Management · Treatment · Bioengineering · Recombinant

M. Kurakula (✉)
Department of Biomedical Engineering, The Herff College of Engineering, The University of Memphis, Memphis, TN, USA
e-mail: mkrakula@memphis.edu

Koteswara Rao G. S. N.
Department of Pharmaceutics, College of Pharmacy, Koneru Lakshmaiah Education Foundation, Vaddeswaram, AP, India

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7.1 Introduction

Probiotics, the growing area of research, are becoming prominent natural resources for the management of several diseases, most importantly with intense focus on cancer prevention and treatment. As per the United Nations World Health Organization (WHO), Food and Agriculture Organization (FAO) defined probiotics as "live microorganisms which, when administered in adequate amounts, confer health benefits on the host" (Joint FAO 2002; Hill et al. 2014). Probiotics are involved in reinforcing the natural defence mechanisms, improving the innate or adaptive immunity, antagonistic effect against pathogenic organisms, with protection from several disorders and diseases. Probiotic with a meaning, "for life", is known to contribute for intestinal balance. In 1984, Hull et al. introduced the first probiotic species *Lactobacillus acidophilus* into research whereas in 1991, Holcombh et al. introduced *Bifidobacterium bifidum* (Tanboga et al. 2003; Bhat et al. 2013).

In the recent times, the application of probiotics is enhancing because of their health promoting effects and prevention and therapy of several diseases, including some types of cancers (LeBlanc 2014). Probiotics can be administered as dosage form or as food product that contains specific viable microorganisms in adequate quantity, which alter the microbiota by implantation/colonization in the host compartment. They play a key role in exerting health benefits to the host (Schrezenmeir and De Vrese 2001). In general, human body is a natural habitat for several strains of microorganisms and the symbiosis with useful organisms seems to be a cause for healthy survival. The content of microflora is influenced by diet, environment, and antibiotics. Tripathi and Giri reported the assumptions and proposals drawn by Elie Metchnikoff that the Bulgarian peasants have long and healthy lives in twentieth century because of consumption of fermented dairy products on daily basis with which the concept of probiotics has been introduced. It was assumed that *Lactobacillus* has protected the intestinal microbiota from other harmful bacteria (Tripathi and Giri 2014; Varzakas et al. 2018). Probiotics are reported to have health benefits in diseases like diabetes, obesity, inflammation, cancer, allergy, infections, etc. Probiotic microorganisms are generally recognized as safe (GRAS), and the commercially used probiotics are predominantly obtained from the safe microorganisms, namely *Lactobacillus* (natural inhabitant in small intestine) and *Bifidobacterium* (in large intestine) (Varzakas et al. 2018). Lactobacilli are the highly used bacteria for food applications rather than Bifidobacteria (Varzakas et al. 2018). Some more microorganisms that are familiar as probiotics include *Lactococcus*, *Streptococcus*, *Bacillus*, *Enterococcus*, *Propionibacterium*, *Saccharomyces*, and *Aspergillus oryzae* (Varzakas et al. 2018; Syngai et al. 2016). Important measures for significant selection of probiotics as treatment aids for health benefits include (1) strain identification, (2) functionality and safety, (3) validated health claims, and (4) proper labelling (Joint FAO 2002). Several approaches for the ideal selection of probiotics have been reported elsewhere (Tripathi and Giri 2014; Varzakas et al. 2018; Syngai et al. 2016; Mitropoulou et al. 2013; Pandey et al. 2015).

7.1.1 Cancer

Cancer is considered as the major cause of deaths worldwide (Torre et al. 2016; WHO 2018). As per the World Health Organization's (WHO) latest reports, low- and middle-income countries are facing 70% of deaths due to cancer. The reasons pertaining to one-third deaths from cancer include high body mass index, consumption of alcohol and tobacco, lack of physical activity, and low intake of fruits/vegetables (WHO 2018). Cancer is said to be a multifactorial disease due to the fact that external factors contribute for 90–95% of the cases, whereas genetic defects contribute for about 5–10% of the cases (Tian et al. 2010). An uncontrolled division of cells caused due to irretrievable DNA damage because of mutations in proto-oncogenes or tumour suppressor genes resulting in increased number of dividing cells is referred to as cancer (Ghany et al. 2015; Saadat et al. 2019). Chemotherapy with anti-cancer drugs with cytotoxicity and immunotoxicity nature may cause patient recovery a challenge and it is driving the research towards the invention of new anti-cancer drugs for targeted therapy with minimal or no side effects (Ghany et al. 2015).

Gut microbiota including the communities of bacteria, virus, and fungi along with their genomes are referred to as gut microbiome. There is a symbiotic mutual beneficial relationship between humans and microbiota resulting in healthy atmosphere for metabolic, immunologic, and motor functions (Blaser 2014; Sharma 2019). A disturbance in the microbiota results in abnormalities of these functions and may also associate with carcinogenesis and cancer-related symptoms (Garrett 2015; Kelly et al. 2016). Cancer or cancer treatment can cause changes in microbiota, which in turn results in progression of disease condition due to disturbance in microbiota (Zitvogel et al. 2017). Restoration and maintenance of beneficial microflora as a part of prevention and therapy for management of several ailments like cancer is an important area of research for the past few decades. Around 10^{14} species of microorganisms are colonized in the digestive tract of human intestine soon after birth (Blaser 2014). Probiotics are known to compete with pathogenic microbiota against adhesion sites, thereby providing stimulation, modulation, or regulation of the host's immune response. This phenomenon can control the disease progression, resulting in improved health (George et al. 2018). An increase in the quantity of beneficial gut bacteria influences metabolism, digestion, immunity, and other functions (George et al. 2018).

7.1.2 Probiotics

Probiotics are reported to have several beneficial effects like regulation of metabolism, immunity, and disorder (inflammatory or functional) and can also offer protection against cancer, infections, diseases, etc. (Mitropoulou et al. 2013; Pandey et al. 2015). Probiotics are rich in fermented dairy products (e.g., yogurt, cultured buttermilk, cheese) and other products like barley, rice, soy, sorghum, maize, wheat, etc. (Kandylis et al. 2016). Probiotics are also rich in breast milk, animal, and human GIT

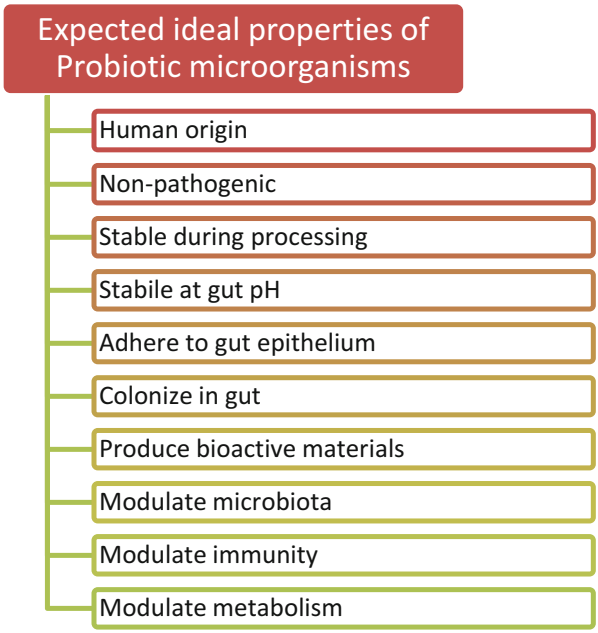

Fig. 7.1 Ideal properties of probiotics (Bhat et al. 2013)

(Syngai et al. 2016). Probiotics are marketed and used as dietary supplements with reported application for prevention and treatment of several diseases (Sanders et al. 2016). Lactic acid bacteria produce several types of natural biopolymers glycans/polysaccharides (carbohydrates) with enormous structural diversity (Bernal and Llamas 2012; Ruas-Madiedo et al. 2002). These bacteria release exopolysaccharides into the surrounding environment or loosely bound to the cell surface (Abedfar and Hossininezhad 2016; Darilmaz and Beyatli 2012).

Probiotics with increased scientific interest have shown an increase in their sales and consumption. These are highly available as functional foods and supplements for therapeutic benefits in several disease conditions. Probiotics are also available in the market as commercial products like tablets, capsules, vials, envelops, etc. with varying doses of microbial compositions (Valdovinos et al. 2017). Several reports are documented supporting the clinical manifestation of probiotics as adjunct therapy in management of diseases like diarrhoe (infectious type, antibiotic associated type), bacterial infections, irritable bowel syndrome, pouchitis, ulcerative colitis, encephalopathy, etc. Specific guidelines and consensus for the use of probiotics in gastroenterology have been documented (Floch et al. 2015; Ritchie and Romanuk 2012; Allen et al. 2011; Hempel et al. 2012; Pillai and Nelson 2008; Dang et al. 2014; Sharma et al. 2008; Wang et al. 2013; Zheng et al. 2013; Ford et al. 2014; Fujiya et al. 2014; Holubar et al. 2010; Valdovinos-García et al. 2019). Ideal properties expected from probiotics are presented in Fig. 7.1.

7.2 Probiotics in Cancer Therapy

Probiotics are not only known for application in treating GIT-related diseases but are also shown to manage the lifestyle diseases like diabetes and life-threatening diseases like cancer. These are also known for management of drug-induced side effects. FAO and WHO working group has released the useful information towards the evaluation of probiotics in food as guidelines (Joint FAO 2002; Vandenplas et al. 2015). Cancer is the major death-causing disease with millions of new cases adding every year and expected to increase enormously by 2030. It is having the unique characteristics of uncontrolled proliferation of cells with capacity to spread to surrounding tissues making it tough to manage (Balducci 2007; Luo et al. 2009; Otake et al. 2006; Hanahan and Weinberg 2011). For the past two decades, extensive research is being done in the area of proving beneficial results in management of cancer on administration of probiotics. It has been established that probiotics are showing promising results in prevention, reduction, and treatment of progression of cancer. Documented results using cell lines, human cancer cells, and in vivo studies are increasing in the research arena. Several types of cancers with beneficial results are noted with respect to colon, rectum, breast, liver, cervix, lungs, etc. (Rossi et al. 2018; Lee et al. 2004; Russo et al. 2007; Orlando et al. 2012; Kim et al. 2008; Alhakamy et al. 2020; Borowicki et al. 2011; Stein et al. 2012; Cousin et al. 2012; Cha et al. 2012; Azam et al. 2014; Ghoneum and Gimzewski 2014). Even though a lot of advancements are coming in the treatment of cancer using nanotechnology and biotechnology concepts, there are still certain limitations like cost and side effects. In this context, the natural preventive and therapeutic measures of using probiotics have become intensive in research field for promoting their application in cancer therapy in addition to other treatments (Gayathri and Rashmi 2016). The need of advancing the in vivo studies and clinical trials for proving the anti-cancer potential of probiotics is increasing with the results obtained from in vitro studies (George et al. 2018). Probiotics are found to be applicable for treatment as well as management of several types of cancers. Singh et al. have reported the results of administration of lyophilized *B. longum* to colon cancer-induced rats where there is a significant suppression of tumour incidence, its progression/proliferation, multiplicity as well as volume (Singh et al. 1997). Effects of probiotics in different types of cancers were well documented in the literature. Probiotics are preferred as adjunct therapies during chemotherapy for cancer patients (Nazir et al. 2018).

Chen et al. reported the beneficial anti-cancer effects of *L. acidophilus* NCFM showing reduction in tumour volume, severity, abnormality and on the other hand enhanced apoptosis of colon adenocarcinoma cells (Chen et al. 2012a). *L. salivarius*, *P. pentosaceus*, and *E. faecium* are found to trigger the synthesis of several fatty acids (short chain, e.g., butyric acid and propionic acid) upon adhering to the human colon cancer cells. This process is found to suppress the proliferation of colon cancer cells and also cause apoptosis of cancer cells (Thirabunyanon and Hongwittayakorn 2013). Anti-tumour effect of *C. butyricum* and *B. subtilis* through improved immunity and attenuation of receptors and transcriptional factors linked with inflammation for protection against colorectal cancer in mice has been reported (Chen et al. 2015).

Liver tumours can be treated with the supplemental therapy of *Bifidobacterium* and also to some extent for treatment of mammary tumours in rats (Reddy and Rivenson 1993).

Considerable suppression of hepatocellular carcinoma in mice has been reported upon treatment with probiotic mixture (*L. rhamnosus*, *E. coli*, *Lactobacillus*, *Bifidobacterium*, *S. thermophilus*) through the secretion of anti-inflammatory agents like cytokines and suppression of Th17 cell differentiation in gut (Li et al. 2016). Fermented milk with probiotic complex has demonstrated reduced proliferation effect on breast cancer cell lines (MCF7) (Biffi et al. 1997). Recurrence rate has been significantly reduced in case of bladder cancer with the administration of *L. casei* (Nanno et al. 2011).

In human myeloid leukaemia cells, there is a significant promotion of TNF-induced apoptosis through NF-κB and MAPK signals modulation upon administration of *L. reuteri* probiotic (Iyer et al. 2008). Thamacharoensuk et al. studied the anti-proliferative and immunomodulatory effect of lactic acid bacteria (five stains) obtained from different sources against Caco-2 cell lines (Thamacharoensuk et al. 2017). It was also reported that the *Lactobacillus*, *Enterococcus*, and *Lactococcus* strains produce metabolites that show enhanced apoptosis of cancer cells resulting in significant anti-proliferation of different cancer cell lines. Several researchers have clearly demonstrated the potential of metabolites from probiotic microorganisms towards suppression of cancer (Haghshenas et al. 2014a, b; Nami et al. 2014a, b, c). Faghfoori et al. have reported the therapeutic potential of different probiotic microorganisms against colon cancer cell lines by downregulation of ErbB-2 and ErbB-3 gene expressions (Faghfoori et al. 2015, 2017). Zununi et al. have demonstrated the molecular mechanism for anti-cancer effect of *Leuconostoc mesenteroides* in colon cancer cell lines (Zununi et al. 2017). Recent clinical studies have also demonstrated the beneficial effects of lactic acid bacteria on different complications (Saadat et al. 2019; Jalali et al. 2019; Montrose and Floch 2005; Venkataraman et al. 2019).

Boursi et al. reported that frequent use of certain antibiotics might contribute to increased risk of cancer in specific organ sites. In their study, 125,441 cases and 490,510 matched controls were included and analysed. The recurrent use of penicillin was claimed to show an increased risk of gastric, oesophageal, and pancreatic cancers (Boursi et al. 2015). Cancer patients undergoing chemotherapy are prone to side effects like infectious complications due to the loss of healthy bacteria and access of pathogenic bacteria to healthy tissues due to disruption of epithelial barriers. Colonizing of pathogenic bacteria causes further complications for a cancer patient. Multi-drug-resistant organisms also prevail and lead to colonization spreading their harmful effects. These infections are of primary reason for worsening the health condition of a cancer patient. Hence, the importance of probiotics to modulate the microbiota is increased. Researchers are showing keen interest in understanding the role of probiotics with established mechanisms and finding out new strategies to make the application of probiotic viable to patients (Galloway-Peña et al. 2017).

7.3 Mechanisms

Several mechanisms were proposed for the effectiveness of probiotics in prevention, treatment, and reduction of cancer progression. A schematic representation of the several proposed mechanisms of action for probiotics in the management of cancer is shown in Fig. 7.2.

Mechanisms of action of probiotics in management of cancer include (Varzakas et al. 2018; Nazir et al. 2018; Faghfoori et al. 2015; Dos Reis et al. 2017).

- Alteration of gut microbiota composition and activity.
- Increased gut barrier functions.
- Production of metabolites, antimicrobials, and anticarcinogens.
- Modulation of immune and inflammatory system in the body.
- Binding and degradation of potential carcinogens.
- Interference with signalling and neuromodulation.
- Protection of intestinal epithelium (from DNA damage).
- Alteration of host physiology.
- Inhibition of proliferation (cancer cells).
- Induction of apoptosis (cancer cells).
- Antioxidant effect.

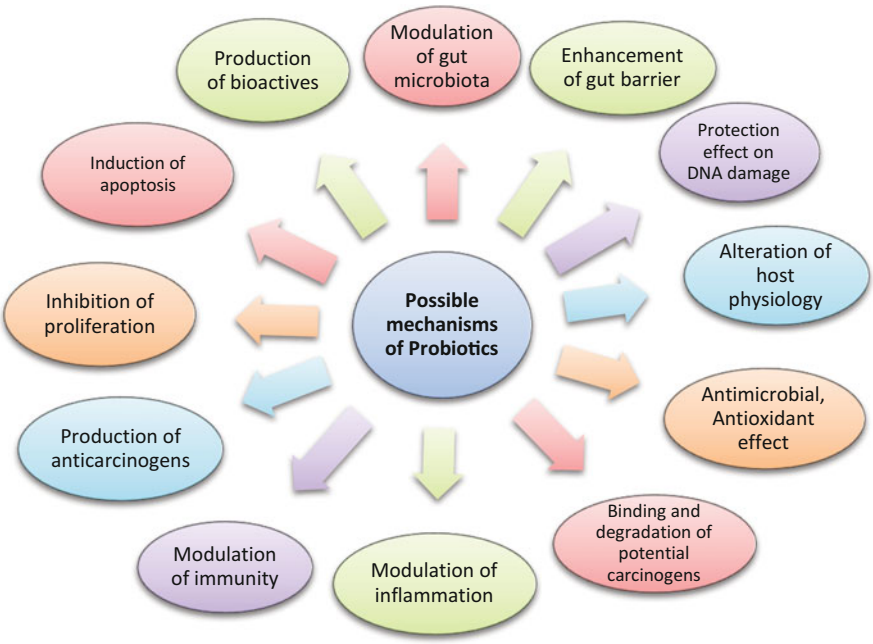

Fig. 7.2 Possible mechanisms of probiotics in management of cancer (Varzakas et al. 2018)

A brief description of the mechanisms with in vitro and in vivo studies has been given below

7.3.1 Modulation of Gut Microbiota Composition and Activity

The important mechanism of probiotics is the modulation of composition of the species of gut microbiota, thereby balancing the beneficial microorganisms. Thus, developed beneficial microbiota can suppress the pathogenic bacteria and their harmful effects. Cancer-inducing bacteria can be suppressed by the beneficial microbiota (Nazir et al. 2018).

7.3.2 Enhancement of Gut Barrier Functions

Gut epithelial has crucial role in maintaining the barrier functions protecting the host from invasion of pathogenic bacteria and toxins. Any alteration in the gut microbiota composition causing dysbiosis associated with pathological conditions disrupts the barrier efficiency of the gut wall. The physiological functions and relationships between epithelial cells and microbiota get disturbed, and the carcinogenic characteristics get enhanced. This disruption may result in induction of inflammatory pathologies causing cancer initiation and thereafter progression (Roy and Trinchieri 2017). Probiotic administration causes enhanced barrier function against the pathogenic bacteria. They upregulate the mucin production of intestine and prevent the translocation of pathogenic organisms (Hardy et al. 2013). They also limit the pathogenic secretions of chloride and water (Brown 2011). Probiotics can increase the gene expressions that favour intestinal barrier integrity (Syngai et al. 2016). Even, probiotics are known for repairing mechanism of damaged epithelial barrier function (Varzakas et al. 2018; Goudarzi et al. 2014). It has been reported that the administration of fermented products of probiotics prevents disruption of intestinal epithelial barrier and inhibits the damage of transepithelial resistance (Commane et al. 2005; Ko et al. 2007). Jones et al. reported the advantage of probiotic LP299v in obstructive jaundice patients with altered gut barrier functions (Jones et al. 2013). It is reported that the probiotics can enhance the protein expression through mucin gene (MUC2 and MUC3) for enhancement of tight junctions that reinforce the gut barrier functions in intestine (Bermudez-Brito et al. 2012). Hence, probiotics are claimed to play a protective role in gut barrier function and maintaining mucus layer integrity (Nazir et al. 2018).

7.3.3 Production of Metabolites, Antimicrobials, and Anticarcinogens

Probiotic microorganisms such as *Lactobacillus* and *Bifidobacterium* have the ability to produce a large number of valuable metabolites like vitamins,

exopolysaccharides, bacteriocins, bioactive peptides, enzymes, fatty acids, etc., which demonstrate several health benefits. These substances differ in their structure and functions resulting in beneficial health factors. They have the ability to show anticarcinogenic properties (Novik and Savich 2020). Like probiotics, the exopolysaccharides obtained from them are also able to show health benefits in cancer and immune diseases (Saadat et al. 2019). It has been reported that the exopolysaccharides obtained from lactic acid bacilli have low cytotoxicity and side effects in comparison to synthetic anti-cancer agents (Ismail and Nampoothiri 2013; Wang et al. 2014). Probiotics show their anti-cancer activity by suppressing the bacteria that produce certain enzymes, which catalyse pro-carcinogens to proximal carcinogens. Examples of enzymes include β-glucosidase, β-glucuronidase, and azoreductase. Probiotics can inactivate nitroreductase enzyme, thereby destroying the carcinogens like nitrosamines and minimizing the risk of exposure to genotoxins (Prasanna et al. 2014). The polysaccharides obtained from probiotics can stimulate immune system components like lymphocytes (T- and B-lymphocytes) and macrophages and induce the release of interleukins showing anti-cancer effect (Ismail and Nampoothiri 2013). These polysaccharides are also able to show biological activities like apoptosis and anti-angiogenesis particularly expression of vascular endothelial growth factor, c-Myc, and c-Fos (Ismail and Nampoothiri 2013). Kim et al. reported that an autophagy protein, Beclin-1, has been regulated by exopolysaccharides obtained from probiotics in addition to relation with apoptosis-related genes (Saadat et al. 2019; Alhakamy et al. 2020). The predominant gram-positive probiotics can produce a good number of antimicrobial agents like acetic acid, lactic acid, and propionic acid. These acids can reduce the intestinal pH, thereby destroying the pathogenic gram-negative bacteria (Šušković et al. 2010). Lactobacilli have shown antagonistic effect against several gram-negative bacteria causing gastric cancer and *Helicobacter pylori*-related strains (Chen et al. 2012b; Kuo et al. 2013). Lactic acid produced from lactobacilli inhibits the growth of *Salmonella enterica* (Makras et al. 2006). Chaikham et al. have found decreased levels of faecal coliforms and clostridia in human intestinal microbial ecosystem (model simulation) with the use of *L. acidophilus* or *L. casei* in their experiments (Chaikham et al. 2012). Many probiotics are known for production of several antimicrobial/inhibitory substances like organic acids, H_2O_2, CO_2, and peptides (lantibiotics, bacteriocins, bacteriolysins) that show protective function against pathogenic organisms (Syngai et al. 2016; Pandey et al. 2015). Administration of probiotics is known to produce anti-inflammatory substances like interleukins, interferons, and cytokines that show effective control over inflammation, which in turn controls carcinogenesis (Le Leu et al. 2005). Several bioactive compounds that are produced by probiotics are shown in Fig. 7.3.

7.3.4 Modulation of Immune and Inflammatory System in the Body

Documented evidences are there for the immunomodulatory effect of probiotics through several studies. Probiotics treatment is known for enhancement of immune

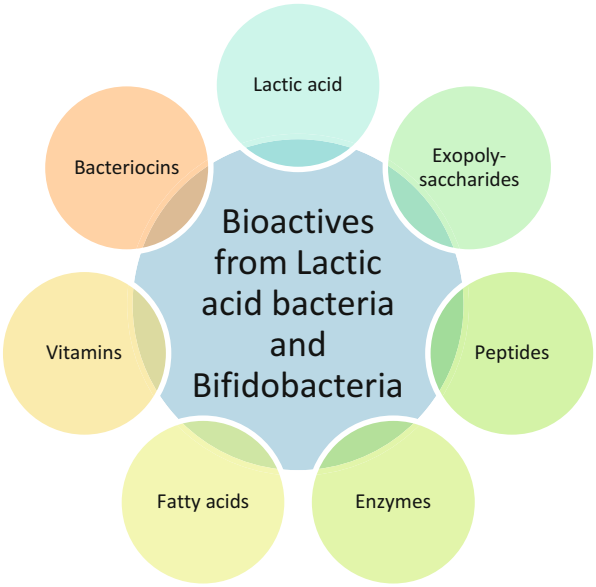

Fig. 7.3 Bioactive substances released from lactic acid bacteria and bifidobacteria (Novik and Savich 2020)

system in cancer patients (Syngai et al. 2016). Li et al. have reported that the use of probiotics has shown enhancement of beneficial bacteria in the gut microbiota composition like *Prevotella* and *Oscillibacter*, which are known for anti-inflammation through producing anti-inflammatory metabolites. The polarization of Th17 is decreased by these metabolites resulting in differentiation of anti-inflammatory cells of gut (Treg/Type 1 regulatory T cells) (Li et al. 2016). Probiotics can show beneficial effects in cancer treatment by inducing dendritic cell maturation and enhancing natural killer cell cytotoxicity (Delcenserie et al. 2008; Takagi et al. 2001; Cai et al. 2016). Probiotics cause upregulation of cytokine secretion (Delcenserie et al. 2008; Azcárate-Peril et al. 2011). Probiotics show beneficial health effects by modulation of immune and inflammatory systems (Martín et al. 2013). With the administration of probiotics, there is a prevention of onset of certain types of cancer (Kato et al. 1994; Aragon et al. 2015; Hu et al. 2015). Different strains of *Lactobacillus* have shown anti-cancer effects as reported in the literature with wide proposal of mechanisms, some of which are not clear (Khazaie et al. 2012; Konishi et al. 2016; Lozano-Ojalvo et al. 2016). In the study conducted by Lakritz et al., it was found that *L. reuteri* has inhibited the carcinogenic severity in mutant mice by immune response triggered CF4+ and CD25+ lymphocytes (Lakritz et al. 2014).

7.3.5 Binding and Degradation of Potential Carcinogens

Colonization of the gut with probiotics causes improvement of host epithelial adhesion capacity, thereby inhibiting the effect of potential carcinogens. Probiotics have the potential of degrading carcinogens like N-nitrosodimethylamine (NDMA) as well as 2-dimethylhydrazine (DMH), which can damage DNA sequence-inducing cancer (Yu and Li 2016). McIntosh et al. have found that *L. acidophilus* strain can control the DMH-induced tumours in large intestine of male Sprague Dawley rats (McIntosh et al. 1999). An adjunct therapy with probiotics has circumvented the effects of 5-fluorouracil like enterocyte apoptosis and declined function of intestinal barrier (Prisciandaro et al. 2012). Probiotics and synbiotics are known for significant decrease in the intestinal procarcinogen enzymes as proved with animal studies associated with colon cancer (Rowland et al. 1998; Nakanishi et al. 2003; De Moreno and Perdigon 2005).

7.3.6 Protection on DNA Damage

Several studies have shown beneficial effects of probiotics like controlling the DNA damage caused by DMH, decreasing the peroxidation of blood lipid, and increasing the total radical tapping antioxidant potential (Park et al. 2007; Ohkawara et al. 2005). Probiotics are also proven for their control over mutagen-induced DNA damage and also against the formation of DNA adduct in cell lines and animal studies (Horie et al. 2003; Oberreuther-Moschner et al. 2004; Yeh et al. 2007; Kumar et al. 2010a).

7.3.7 Inhibition of Proliferation of Cancer Cells

Several other studies have shown that the treatment with *L. casei* caused enhancement of NK and T cells improving the phagocytosis nature of macrophages, thereby inhibiting the progression of different types of cancer in mice (Takagi et al. 2001; Yamazaki et al. 2000; Foo et al. 2011). Treatment with *Bacillus polyfermenticus* has shown stimulation of IgG production with modulation of CD4þ, CD8þ, or NK cells in cancer patients (Rossi et al. 2018).

7.3.8 Binding and Degradation of Potential Carcinogens

Probiotics compete with the pathogenic bacteria for available receptors, nutrients, and growth factors, thereby suppressing the pathogens (Kahouli et al. 2013). Lactic acid bacteria were found to reduce the DNA damage, very effectively, caused due to chemical carcinogens as proved by animal models of colorectal cancer (Kahouli et al. 2013; Uccello et al. 2012). Not only the oral administered probiotics, there are reports with other routes, for example, intranasal administered probiotics has

demonstrated anti-cancer effects of *L. casei* in virus-induced cancer of mouse model (Lozano-Ojalvo et al. 2016; Bermudez-Humaran et al. 2018). Lactic acid-producing probiotics exert antimicrobial effect on pathogens with the reduction of gut pH. Some may interfere with quorum sensing, which is more responsible for virulence nature of the bacteria (Asad and Opal 2008). Enhanced mucin production due to probiotics serves as the antibacterial barrier preventing the binding and thereby invasion of pathogens. Probiotics also promote the secretion of IgA in gut, which binds to pathogens and clear them. They also exert anti-inflammatory effect in the gut by inhibiting the NF-kB and IL-8 factors. It has also been reported that some probiotics function by activating the opioid and cannabinoid receptors in gut for application in irritable bowel syndrome (Mizock 2015).

Probiotics in adequate number can consume the available monosaccharides in surroundings, thereby resulting in the depletion of sources for pathogenic organisms like *Clostridium difficile* as they solely depend on monosaccharides. This sort of mechanism can also reduce the prevalence of pathogenic bacteria (Wilson and Perini 1988). Induction of several types of cancers due to translocation of pathogenic bacteria through the disrupted epithelial barrier in gut has been answered by probiotics application. The attachment of pathogenic *E. coli* over the gut wall can be inhibited by the use of probiotics (Wilson and Perini 1988; Naveen et al. 2020a). Probiotics can also enhance the gene expression (signalling for E-cadherin and β-catenin) for developing tight junctions to re-establish the gut barrier integrity. *Lactobacillus* is known to exclude pathogens from mucous by providing competitive adhesion (due to surface adhesins) and merging with mucin secretions of intestinal epithelial cells (Bermudez-Brito et al. 2012).

7.3.9 Interference with Signalling

In cancer patients, probiotics have a significant influence on cell signalling. *L. reuteri* has been reported to cause downregulation of NF-kB-dependent genes. This in turn controls the proliferation (Cox-2, cyclin D1) of cancer cells and also their survival (Bcl-2, Bcl-xL) (Lee et al. 2008).

An inhibition of protein kinase signalling pathway (p38 mitogen-activated) has been reported upon probiotics administration to patients with colorectal carcinoma (Liu et al. 2012). Inflammation through several pathways may contribute for cancer progression through signal transduction, activation of transcription 3 (STAT3) followed by nuclear factor-kB (NF-kB), genotoxicity, tissue damage, invasion, proliferative responses, and metastasis (Elinav et al. 2013). In a cancer-induced rat associated with colitis, the administration of *Lactobacillus* and VSL#3 probiotics has shown reduced and delayed transformation of inflammation to dysplasia (Matsumoto et al. 2009; Naveen et al. 2020b). Several probiotics are known to release certain compounds that can inhibit pathogens' signalling character of sensing the quorum, thereby preventing the bacterial toxicity (Brown 2011; Goudarzi et al. 2014). Probiotics can stimulate the adaptive and innate immunity of host for better immune responses (Bermudez-Brito et al. 2012). Kim et al. reported to have studied

the influence of *L. casei* on innate immune response using mice animal model. The reports have shown significant improvement in the immunity via phosphorylation of several signalling pathways notably p65, p3, NF-kB, MAPK, and MAPKAPK-2 (Kim et al. 2006).

7.3.10 Anti-Oxidant Effect

Anti-cancer effect of probiotics can also be attributed to the productions of antioxidants (SOD, CAT, and GSH) (Dasari et al. 2017).

7.4 Probiotics in Lung Cancer

Lung cancer is considered as the deadliest disease and most prevailing cancer in the world for both men and women. Lung cancer has been reported as the frequently occurring and high mortality causing disease (Torre et al. 2016, 2015; Toyoda et al. 2008; Sharma et al. 2018). Lung cancer is broadly classified into two types: namely small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). SCLC accounts for 18% cases, which grows faster and shows metastasis with wider spread to other organs of body. NSCLC accounts for 78% of cases, which spreads slowly. NSCLC is further classified as adenocarcinoma (40% and most prevailing), squamous cell carcinoma (25%), and least occurring type as large cell lung cancer (10%). Schematic representation of the types of lung cancers is shown in Fig. 7.4 (Toyoda et al. 2008).

Specific causative factors for lung cancer include smoking, genetic factors, environmental factors, heavy metal consumption, alcohol intake, respiratory complications, exposure to radon gas, silica dust, asbestos, and several elements (Lu et al. 2013; Vineis et al. 2006; Zhang et al. 2012; Druesne-Pecollo et al. 2014; Islami et al. 2015). Among all, smoking is reported to be the major cause of lung cancers (Alberg et al. 2013; Kim et al. 2014). Lung cancer is associated with malignant proliferation, invasion, and metastasis (Hirsch et al. 2017). Survival rate

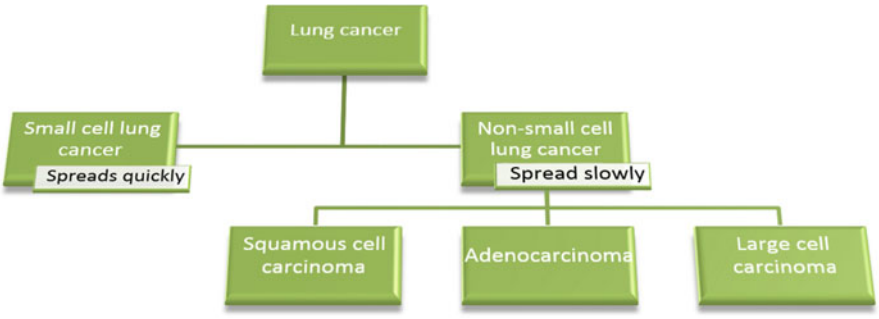

Fig. 7.4 Classification of lung cancers (Toyoda et al. 2008)

of patients suffering with lung cancer is very less counting as 21% for women and 15% for men as per American Cancer Society, 2017 statistics. However, improvement in health condition can be seen with early stage lung cancer patients (American Cancer Society 2017). For early stage lung cancer, surgery is the remedy; however, for advanced cases, the severity of the disease needs multiple remedies like systemic chemotherapy or immunotherapy (Howington et al. 2013; Johnson et al. 2014; Wakelee et al. 2014). It has been found that there is a need of innovative strategies to prevent, manage, and treat the lung cancer. The "World Cancer Research Fund" and also the "American Institute for Cancer Research" have observed the vital roles of nutrition, food, lifestyle, physical activity, and body factors in the prevention and management of several types of cancer (World Cancer Research Fund and American Institute for Cancer Research 2007). These systemic chemotherapies show several side effects keeping the patient in more unwanted problems. They may further worsen the patients' immunity, strength, and treatment response and even make the overall treatment more expensive (Bradbury et al. 2017; Andreyev et al. 2014; Marx et al. 2016; Tong et al. 2009; Tohyama et al. 2013; Verma et al. 2016). It was noted that there is an axis between the microbiota of gut and lungs indicating the chances for impact of changes in gut microbiota on lung condition (Bingula et al. 2017). Normal microflora is required for the maintenance of immune homeostasis, nutrient utilization, health benefits, resistance against infectious pathogens, regulation of host metabolism, and normal functioning of the systems (Brestoff and Artis 2013; Hooper et al. 2012; Iida et al. 2013; Zhang and Sun 2018). Any disturbance in the microflora may lead to more susceptibility for disease incidence and progression of diseases like obesity, malnutrition, asthma, inflammatory bowel syndrome, diarrhoea, psychiatric problems, cancers, etc. (Yu and Li 2016; Sharma et al. 2018). Even though the microbial count in lungs is quite small compared to the gut microbiome, an imbalance in the microbiota of lung results in several pulmonary/respiratory diseases even leading to cancer. A recurrent use of antibiotics may cause damage to the microbiota of lungs and can increase the risk of lung cancer (De Steenhuijsen Piters et al. 2015; Schreiber et al. 1972). Due to the increased mortality rates with cancer incidences, research has taken high priority in the prevention and treatment strategies for different types of cancers. In addition to advanced treatments, scientists are also looking for alternative therapies involving lifestyle modification, physical activity, and nutrition supplement in order to make the patient suffer from less side effects. As a positive result, probiotics have been reported extensively in the management of several disease conditions including cancers like colon, blood, breast, rectum, cervical, prostate, skin, oesophagus, liver, bladder, gall bladder, head, and neck (Dasari et al. 2017; Kumar et al. 2010b).

Even though the claims for probiotics application in disease management have been increased tremendously, the exact mechanism of action is still under search. The possible principles for antimicrobial and anti-tumour effects of probiotics have been presented in the literature as competitive binding of pathogens, degradation of causative elements, modulation of gut microbiota and immune response, modulation of translocation of pathogens, release of metabolites or substances that have specific functions, etc. cumulatively resulting in delayed tumour growth, increase in survival

time, reduction in chemotherapy-induced side effects, post-operative complications, etc. (Sharma et al. 2018; Kumar et al. 2010b; Raman et al. 2013).

Several in vitro cell line studies and in vivo animal/human studies were performed to assess the application of probiotics in cancer management that too particularly in lung cancer cases.

Cheng et al. have reported that excess antibiotic usage has shown more susceptibility for development of engrafted B16/F10 melanoma as well as Lewis lung carcinoma with short survival rate due to imbalanced lung microbiota. The antibiotic-treated mice have shown more aggressive and larger tumour development in lungs due to the loss of immune system and impaired mechanisms. The reason was attributed to the defective induction of γδT17 cell response and established tumour microenvironment in the antibiotic-treated mice lungs. For restoring the impaired immune surveillance phenotype, inclusion of normal γδT cells or IL17 cells has been done. This study has once again clearly supported the potential of commensal bacteria in maintaining immune homeostasis (Cheng et al. 2014). There is an association between the oral diseases and the lung cancer risk. The biomarkers *Capnocytophaga* and *Veillonella* are reported to be the most prevailing organisms in saliva of patients associated with lung cancer (Yan et al. 2015). Pharmacokinetics and pharmacological profile of anti-cancer drugs are influenced by the microbiota of gut and hence probiotics have their effect. More intense research is necessary to understand the role of probiotics on modulation of bacterial enzyme activity during the chemotherapy for cancer patients (Maleki et al. 2015). Zamberi et al. reported the application of fermented milk product containing the probiotic, Kefir, using 4T1 breast cancer cells. Studies in BALB/c mice revealed the cytotoxic effects of Kefir. Kefir has significantly improved the T helper cells and cytotoxic T cells with significant reduction in the metastasis to lung and bone marrow (Zamberi et al. 2016).

Among several side effects caused by chemotherapy, diarrhoea is one major problem caused by intestinal epithelial cell apoptosis or alteration in intestinal microflora or dysfunction of intestinal barrier or production of proinflammatory cytokines (Touchefeu et al. 2014). In a most recent literature, it was reported that Yang et al. have selected lung cancer patients and administered *C. butyricum* as per the pre-planned protocol and analysed the flora in faecal matter at different time intervals during chemotherapy. They also did placebo trials for comparison. The results indicated that *C. butyricum* treatment has reduced the chemotherapy-induced diarrhoea in lung cancer patients, reduced the inflammatory response, and also encouraged the maintenance of homeostasis (Tian et al. 2019). Bingula et al. have reported the clinical trial study protocol for elaborate understanding of the gut, lung, and upper airways microbiota in NSCLC patients. It is an observational study and analysed the influence of chemotherapy and local microbiota population over lung cancer (Bingula et al. 2018). The presence of dysbiosis or malignancy in lung is associated with dynamic interaction of various factors related to immune, microbial, and environmental. IL-6 and IL-8 are expressed in lung cancer cells (premalignant and senescent) and elevated in the inflammatory stress conditions of lungs. These conditions are causative for progression towards lung cancer through their direct

effect on stimulating NF-kB-1 pathway in lung epithelial cells (Lin and Karin 2007; Davalos et al. 2010).

Matsuzaki et al. have reported the first evidence of probiotic effect on lung cancer and thereafter the research progressed with promising outcomes. In 1985, they reported the anti-tumour activity of *L. casei* by conducting in vivo studies using Lewis lung carcinoma cells and line-10 hepatoma in the animal models of syngeneic mice and guinea pigs. The strains of *L. casei* were found to suppress the pulmonary and regional lymph node metastases (Matsuzaki et al. 1985).

In 1991, Kim et al. have studied the application of seven strains of probiotics (*Lactobacillus casei*, *L. plantarum*, *L. acidophilus*, *L. bulgaricus*, *Leuconostoc mesenteroides*, *Streptococcus thermophilus*, and *Bifidobacterium bifidum*) in sarcoma 180 cells and mouse Lewis lung cancer cells. The probiotics were administered intraperitoneally, and the anti-tumour effects were measured through increase in body weight and mean survival time. Among all, *L. casei* has shown promising results (Kim et al. 1991). Several trials with conventional therapies looking for the dysbiosis and comparing with probiotic supplementation were done with positive results. Chemotherapy effect on microbiota and the combination therapy with probiotic were assessed for the understanding of importance of probiotics in lung cancer therapy (Kelly et al. 2001).

In case of metastatic Lewis lung carcinoma (3LL) and solid sarcoma 37 (S37) models, there is an inhibition of metastasis with the administration of anti-tumour vaccine, cytotoxic lectin obtained from *B. subtilis* B-7025 along with the probiotic mixture (*S. cerevisiae* 14K and *E. faecium* K-50 or their metabolites). The application of combination therapy has shown synergistic effects in the treatment of cancer-induced animal models with a 2–2.5-fold metastasis inhibition compared with the animals that received only vaccine (Tanasienko et al. 2005). Vetizou et al. have found the potential of *Bacillus thetaiotaomicron* or *B. fragilis* species in stimulating the immune response and increasing the performance of CTLA-4 antibodies in sarcoma tumour growth in mice model. A control of antibiotic-treated mice (germ free) was studied, where the CTLA-4 antibodies are non-reactive in the sarcoma tumour. Hence, the application of gut microbiota for better immune response and therapeutic benefit was reported (Vétizou et al. 2015).

Aragon et al. reported the anti-tumour effect of *L. casei* strain-fermented milk on breast cancer-induced mouse where there was suppression of tumour growth showing less tumour vascularity and extravasation and decreased metastasis to lungs. Decreased infiltration of macrophages into both lungs and tumour with modulation of immune response (enhanced CD8+ and CD4+ lymphocytes) resulted in suppressed growth of tumour (Aragon et al. 2015). Han et al. have demonstrated the probiotic effect of *L. lactis* on various cancer cell lines (human lung carcinoma [SK MES1-KCLB 30058], human colon adenocarcinoma [DLD 1-KCLB 30058, HT 29-KCLB 30038, and LoVo-KCLB 10229], and human breast adenocarcinoma [MCF 7-KCLB 30022]). The results have shown promising anti-cancer and also anti-inflammatory activity of the selected strain with strong inhibition of cancer proliferation at a level of 10^6 CFU/well. It has also shown decreased production of nitric oxide (NO) as well as pro-inflammatory cytokines. These results encouraged

the recommendation of probiotics in management of lung cancer as well as in other cancers (Han et al. 2015). Mlu et al. have reported the advantage of using probiotics along with chemotherapy stating that the combination therapy has shown improved intestinal microflora, thereby decreasing the complications of gut in lung cancer patients. The study has given positive results with the administration of *Lactobacillus*, *Bifidobacterium*, and *Bacteroides* along with chemotherapy (Mlu et al. 2013).

Influence of probiotics on Lewis lung cancer (LLC C57BL/6J)-induced mice has been demonstrated by Gui et al. Three groups of mice were administered with cisplatin, cisplatin/ABX (combination of vancomycin, neomycin, and ampicillin) and a combination of cisplatin/probiotic (*Lactobacillus acidophilus*). The researchers found reduction in tumour size and also an increase in the survival rates for the combination of cisplatin with probiotics rather than the chemotherapy alone. The positive effect of probiotic supplementations was also attributed with upregulation of interferon-γ, Gzmb, and Prf1mRNA expression. From western blotting studies, it was also noted that the probiotic strain has shown decreased expression of Vegfa and Ras oncogenes whereas increased expression of Cdkn1b and Bax tumour suppressor genes (Gui et al. 2015). Lee et al. have demonstrated the anti-cancer effect of *L. lactis* KC24 on different cancer cell lines like lung carcinoma (SK-MES-1) along with studies on colon carcinoma (HT-29 and LoVo), as well as breast carcinoma (AGS and MCF-7). Results have shown a significant anti-proliferative effect on all cell lines at a level of 10^6 colony-forming units/well of *L. lactis* KC24. With MTT assay, a strong inhibition of proliferation was noted (Lee et al. 2015). Microbiota in lungs provides resistance to colonization of respiratory pathogens and also provides immune tolerance. Le Noci et al. revealed that the modulation of pulmonary microbiota by aerosolization of antibiotic or probiotic decreases tumour growth in lungs. Antibiotic or probiotic aerosol showed improved chemotherapy against experimental metastasis (Le Noci et al. 2018). In lung adenocarcinoma model, the eradication of microbiota due to cyclophosphamide chemotherapy has also shown influence on the immunomodulation due to reduced levels of immune cells (Sistigu et al. 2011). Cisplatin treatment alone and with probiotics study conducted by Gui et al. has clearly presented the promising role of probiotics as anti-tumour agents by decreasing the tumour size and increasing the survival rates of mice with Lewis lung cancer (Gui et al. 2015).

Sivan et al. have studied the application of oral administration of cocktail of *Bifidobacterium* (*longum*, *lactis*, and *breve*) in cancer-induced mouse. The results have shown abolishment of tumour outgrowth by promoting anti-tumour immunity and facilitating anti-protein 1 or its ligand 1. With *Bifidobacterium* species administration, an improvement is found with immune responses like T cell activation and co-stimulation (CD8+), interaction of cytokine–cytokine receptors, improved function of dendritic cells, upregulation of 760 genes, and chemokine-linked transmission of immune cells to tumour microenvironment (Sivan et al. 2015). In further studies it was reported that chemotherapy of lung cancer caused loss of microbiota in lungs with modifications in treatment strategies like multi-drug therapies and dose variations. It was noted that the chemotherapy has influence on the microbiota, which in turn is worsening the cancer condition (Gui et al. 2015). With these

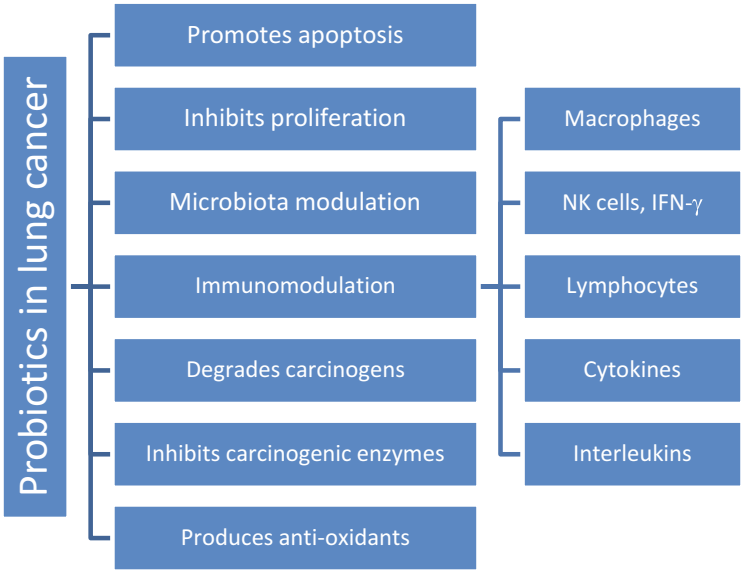

Fig. 7.5 Role of probiotics in lung cancer treatment (Sharma et al. 2018)

preliminary extensive studies, researchers have established the role of microbiota in management of lung cancer. With the established axis between the gut and lung microbiota, use of certain markers has been introduced for the study of role of gut microbiota in handling lung cancer.

Daillere et al. found that the advanced lung cancer patients under chemotherapy have shown immune responses (specific memory Th1 cell) with prolonged progression-free survival upon administration with *Enterococcus hirae* and *Barnesiella intestinihominis* that have been represented as "oncomicrobiotics". These agents are also known to enhance the performance of alkylating immunomodulatory agent (Daillère et al. 2016). Na-Kyoung et al. studied the effect of *L. lactis* (isolated from Kimchi) against different cancers like gastric carcinoma, colon carcinoma, breast carcinoma, and lung carcinoma. This probitic has inhibited the adhesion of six pathogens to mucus layer and has shown competition for intestinal adherence. It has also shown significant anti-oxidant effect in addition to the promising anti-cancer effect (Lee et al. 2015). The established reports clearly explain the role of some of the probiotics as beneficial microbiota that act on immune system and strive for the eradication or reduced progression of cancers.

An overview of the role of probiotics in lung cancer treatment and the studies (in vitro and in vivo) reported in literature are shown in Fig. 7.5 and Table 7.1, respectively.

Table 7.1 In vitro and in vivo studies conducted for probiotics in lung cancer

In vivo studies		
Probiotic	Animal model	Reference
Lactobacillus casei CRL 431	Mice, BALB/c	Aragon et al. (2015)
Commensal microbiota	Mice, LLC C57BL	Cheng et al. (2014)
Kefir	Mice, BALB/c	Zamberi et al. (2016)
Lactobacillus casei—YIT 9018, *Propionibacterium acnes* C7	Male mice, C57BL/6, Guinea pigs	Matsuzaki et al. (1985)
Lactobacillus acidophilus, *L. bulgaricus*, *L. casei*, *L. plantarum*, *Leuconostoc mesenteroides*, *Streptococcus thermophilus*, and *Bifidobacterium bifidum*	ICR mice, sarcoma 180 cells, mouse Lewis lung carcinoma (LLC1) cells	Kim et al. (1991)
Saccharomyces cerevisiae, *B subtilis* B-7025, *Enterococcus faecium* K-50	Mice, BALB/c and C57B16; solid sarcoma 37 (S37), and metastatic Lewis lung carcinoma (3LL)	Tanasienko et al. (2005)
Bacteroides fragilis	Mice	Vétizou et al. (2015)
Bifidobacterium infantis	Mice, LLC C57BL	Zhu et al. (2011)
Bifidobacterium infantis	Mice, LLC C57BL	Li et al. (2012)
Lactobacillus, *Bifidobacterium*, and *Bacteroides*	Lung cancer patients	Mlu et al. (2013)
Lactobacillus acidophilus	Male mice, C57BL/6J	Gui et al. (2015)
Lactobacillus rhamnosus	Mice, C57BL/6	Le Noci et al. (2018)
Bifidobacterium cocktail (*longum*, *lactis*, and *breve*)	Mice, C57BL	Sivan et al. (2015)
Barnesiella intestinihominis, *Enterococcus hirae*	Mice, C57BL and cancer patients	Daillère et al. (2016)
Bifidobacterium longum	Male nude mice, BALB/c mice	Fu et al. (2005)
Clostridium butyricum	Lung cancer patients	Tian et al. (2019)
Mixed probiotics	Cancer patients	Ciernikova et al. (2017)
In vitro studies		
Probiotic	Cell lines	Reference
Lactococcus lactis NK34	SK-MES-1, DLD-1, T-29, LoVo, AGS, and MCF-7 cells	Han et al. (2015)
Lactococcus lactis KC24	MRC-5 cells (human lung cell line, KCLB 10171), SK-MES-1 cells (human lung carcinoma cell line, KCLB 30058)	Lee et al. (2015)
Lactococcus lactis KC24	Lung carcinoma (SK-MES-1) cells	Lee et al. (2015)

7.5 Recombinant Probiotic in Cancer

As an advancement, the probiotic *B. longum* strain was loaded with the endostatin gene (*B. longum*) and administered orally to tumour-bearing nude mice via drencher preparation. The results have shown the strong inhibition of growth of solid tumour in liver of nude mice and also the survival rate was increased. An additional supplement with selenium has further shown promising results due to the activity of NK and T cells with stimulation of IL-2 and TNF-α in BALB/c mice. Hence, it was found that *B. longum* could be used as a specific vector for the transportation of anti-cancer genes in gene-based cancer therapy (Fu et al. 2005). Zhu et al. have established *B. infantis*-mediated sFlt-1 gene transferring system (recombinant therapy) using electroporation for studying the anti-tumour effect on Lewis lung cancer in mice model. sFlt-1 is the soluble FMS-like tyrosine kinase receptor, an extra membrane part of vascular endothelial growth factor receptor-1 (VEGFR-1) that has anti-tumour effects. Tumour growth was significantly inhibited showing prolonged survival time of mice. The mechanism is attributed to the successful expression of sFlt-1 at gene and protein levels, which inhibited the VEGF-induced growth of human umbilical vein endothelial cells. Hence, this approach was claimed to be a promising model for treatment of lung cancer (Zhu et al. 2011). Another approach reported was the use of recombinant *Bifidobacterium infantis*. A prokaryotic expression system was established using *B. infantis*-mediated soluble kinase insert domain receptor (sKDR). Lewis lung cancer mice models were used for the study. *B. infantis* containing the plasmids pTRKH2-PsT and pTRKH2-PsT/sKDR was used in the study in addition to control group. Quality of life and survival time were recorded. The group treated with recombinant *B. infantis* containing pTRKH2-PsT/sKDR plasmid has shown improved suppression of tumour growth compared to other groups. There has been a noted necrosis rate of the tumour and prolonged survival time. In vitro MTT assay was also performed for the observation of anti-angiogenesis effect (Li et al. 2012).

7.6 Safety Considerations

Very rare reports are there concerning the side effects of probiotics usage like gastrointestinal distress (e.g., bloating) and are also possible only in immunocompromised groups like pregnant women, new-born, and geriatrics (Szajewska et al. 2010). There are reported concerns with *Lactobacillus* resistance against vancomycin and the possible transfer of such resistance to pathogens in gut (Varzakas et al. 2018; Saulnier et al. 2009). Probiotics are well known for safety primarily with respect to lactobacilli and bifidobacteria. Probiotics are preferred when they come under GRAS category; however, there is every chance that they provoke side effects based on the host susceptibility. Very low rate of systemic infection around 0.05–0.40% was observed with epidemiological studies on probiotics use. Administration of probiotics in late pregnancy and early infancy was also reported to be considered as safe; however, those containing hidden allergens need to be taken care

(Allen et al. 2010; Martín-Muñoz et al. 2012). There are reported cases of invasive infections due to probiotics application with immunocompromised patients (Borriello et al. 2003; Mackay et al. 1999; Rautio et al. 1999). Antibiotic resistance may be the possible factor upon long-term usage of probiotics with antibiotic therapy and even sepsis has been reported in children with short gut. The resistance might transfer to other bacteria too (Rautio et al. 1999). However, the safety concerns vary from strain to strain, and the potential benefit of probiotics should be weighed against the risk caused by them, if any (Redman et al. 2014). Gargar and Divinagracia reported the cases found with bacteraemia from probiotics administration, i.e., *Bacillus clausii* (Gargar and Divinagracia 2019).

7.7 Future Prospectus

With the widespread knowledge and application of probiotics in the prevention and treatment of several diseases including cancer, there seems to be a lot of scope for their application in future. The enormous research going on with relation to probiotics in cancer, and their findings are giving positive approach for their supplementation with chemotherapy or immunotherapy. However, still better understanding of the specific strains of bacteria responsible for improvement in particular disease condition needs to be established in a scientific manner. Dosage regimens of probiotics need to be developed on case-by-case basis.

7.8 Conclusion

With the huge number of promising results found in the literature, it is concluded that probiotics are going to be the attractive alternative or the promising supplementation for the prevention and treatment of several cancer ailments as they are life threatening. Lung cancer also has improvement with the administration of probiotics and has proven reports with advantage over side effects during chemotherapy. The future may rely on the identification and formulation of an optimum cocktail of probiotics that suit the required treatment strategy substituting the conventional therapies. Such strategies with probiotics administration might bring more benefit with less risk particularly in case of cancer management. Even though a lot of innovative strategies are being introduced in chemotherapy, the side effects are also in the lane of worsening the patient condition, hence there is every search for new treatments. In this context, the safe application of probiotics has gained valuable importance in handling the different types of cancers. The recombinant approaches using probiotics as vectors are still promising to provide the attractive results in the treatment of cancers. The application of microbiota has been driven from keeping the host normal well-being to a level of management of several types of cancers. Lung cancer is one of the prevailing deadly disease. Probiotics role in the prevention and treatment of lung cancers is witnessing successful results, and their mechanism of actions has been developed for complete scientific understanding. The

modulation of microbiota in gut has been found to be responsible for proper maintenance of good respiratory condition and healthy lungs.

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8 Probiotics for Prophylaxis and Management of Breast Cancer: Preclinical and Clinical Evidence

Sheyda Ranjbar, Seyed Afshin Seyednejad, Shahab Edalatian Zakeri, Hossein Rezaeizadeh, and Roja Rahimi

Abstract

There is solid evidence regarding the role of gut microbiota in various types of cancer. Particularly, the gut microbiota is associated with breast cancer (BC) via the immune- and estrogen-mediated pathways. Besides, there is evidence regarding the interactions between BC incidence and dysbiosis. Probiotics are beneficial microorganisms, which can manipulate gut microbiota composition and function through different mechanisms. In the light of these facts, modulation of gut microbiota via consumption of the probiotic products may hold promise in the prevention and treatment of BC. In this chapter, the authors go through the literature and present studies in human and animal models on the role of probiotics in the prevention and treatment of BC and the underlying mechanisms. Besides, the shortcomings of the current state of research and translational

S. Ranjbar · S. E. Zakeri
Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
e-mail: sh-ranjbar@farabi.tums.ac.ir; sh-edalatianz@student.tums.ac.ir

S. A. Seyednejad
Department of Toxicology and Pharmacology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
e-mail: sa-seyednejad@student.tums.ac.ir

H. Rezaeizadeh
Department of Persian Medicine, School of Traditional Medicine, Tehran University of Medical Sciences, Tehran, Iran
e-mail: rezaeizadeh@tums.ac.ir

R. Rahimi (✉)
Department of Traditional Pharmacy, School of Traditional Medicine, Tehran University of Medical Sciences, Tehran, Iran
e-mail: rahimi_r@tums.ac.ir

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challenges of extrapolating the *in vivo* results to the clinical outcome are addressed. Furthermore, potential favorable effects of probiotics consumption during the chemotherapy in BC patients are covered. Safety concerns and regulatory considerations alongside the present trend in the probiotics market are also reviewed. In the end, the prospects of probiotics administration in BC are discussed.

Keywords

Gut microbiota · Probiotics · Breast cancer · Estrogen · Pro-inflammatory cytokine · Lactic acid bacteria · Lactobacillus

8.1 Introduction

Breast Cancer (BC) is the most prevalent cancer and the leading cause of cancer death in women worldwide, with approximately 2.1 million new cases and more than 0.6 million deaths in 2018 (Bray et al. 2018). This corresponds to an age-standardized incidence rate of 46.3 cases per 100,000 women, and an age-standardized mortality rate of 13 deaths per 100,000. BC is generally categorized based on the presence or absence of three receptors indicated in the course of this disease: estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor-2 receptors (HER2). Most therapeutic regimens target these receptors. BC is a complex and multifactorial disease and despite enormous efforts put into research, the exact etiology is still unknown. A multitude of factors may influence the development of BC but extended exposure to estrogen appears to be the most important one (Travis and Key 2003).

The human microbiome is the collective genetic material of all the microbes residing in and on the human body. In recent years, there have been major advances in our understandings of human microbiome and microbiota—an ensemble of microorganisms found on and within human—owing to sublime progress in deep sequencing technologies (Navas-Molina et al. 2013). Various studies have scrutinized and linked the microbiome composition and dysbiosis—microbiota imbalance—to the occurrence of several cancers (Sheflin et al. 2014). BC is one of these malignancies that have gained considerable attention due to both its epidemiologic importance and strength of evidence related to the pathological role of microbial imbalance (Fernández et al. 2018; Plaza-DÍaz et al. 2019).

Probiotics are products containing live nonpathogenic microorganisms, which can regulate the composition and function of gut microbiota and have proven to be beneficial in various pathological conditions, including cancer (Nazir et al. 2018; Górska et al. 2019). In light of these features of probiotics and considering the role of gut microbiota and dysbiosis in BC, consumption of probiotics appears as a realistic research angle in the search for BC prophylaxis and treatment. Accordingly, research regarding the role of probiotics consumption in the prevention and treatment of BC

has gained much interest in recent years and have been the subject of various clinical and preclinical studies that are going to be reviewed in this manuscript (Ranjbar et al. 2019).

8.2 Association of Gut Microbiota and BC

8.2.1 Cross-Talk Between Gut Microbiota and the Immune System

The relationship between gut microbiota and BC is multi-faceted. One of the main characteristics that relates gut microbiota to BC is the existing cross-talk between gut microbiota and the immune system. Although the immune system is the main protective sword to combat cancer, it can also contribute to tumor initiation and progression. It is well-established that chronic inflammation can induce uncontrolled innate and adaptive immune responses which can eventually lead to alterations in host cell proliferation and cell death signaling pathways leading to tumorigenesis (Goodman and Gardner 2018). In particular, persistent inflammation is associated with high risks of BC; inflammation can increase the production of prostaglandin E2 (PGE2) by upregulation of cyclooxygenase 2 (COX2), which can facilitate the conversion of androgen precursors to estrogen, contributing to the occurrence of ER^+ BC (Gierach et al. 2008; Subbaramaiah et al. 2012).

Gut microbiota regulates intestinal immunity by adjusting the regulatory and effector immune cells as well as the cytokine profile of the intestine environment, ultimately determining the pro/or anti-inflammatory response (Mishima and Sartor 2019). Any imbalance in the composition of gut bacteria, which is referred to as dysbiosis, due to aging, medications, nutritional and lifestyle changes, can cause inflammation in the gut by downregulation of regulatory T cells (Tregs) and dendritic cells (DCs) and rise in the pro-inflammatory cytokines, which is mostly triggered by the endotoxin of gram-negative bacteria (MacDonald and Wagner 2012). Based on the mentioned role of inflammation in tumorigenesis, dysbiosis can contribute to tumor initiation by increasing the population of bacterial strains, which can induce pro-inflammatory responses in the gut. However, it should be noted that the mentioned cross-talk between gut bacteria and the immune system is not confined to the intestine. This systemic cross-talk is mostly attributed to the intestinal mucosal barrier which is responsible to protect the body from exogenous antigens. In particular, peyer's patches expose the exogenous antigens, including those related to the gut microbiota, to the immune system and thus promote a systemic immune response by antigen-mediated activation of T and B cells which can freely migrate to distal organs such as mammary glands to exert immune responses (Shapira et al. 2013). This systemic immune response, provoked by resident intestinal bacteria, elucidates the link between gut microbiota and distal cancers such as BC and can explain the reciprocal interactions observed between dysbiosis and BC (Fernández et al. 2018). In fact, studies show that dysbiosis is associated with increased risk of BC and also BC patients were found to have altered

gut microbiota composition (Goedert et al. 2015; Plaza-DÍaz et al. 2019). In addition to the mentioned systemic immune response, there is evidence regarding the translocation of gut microbiota to mammary glands via an endogenous pathway (Jost et al. 2014). Although this hypothesis has not been fully clarified, this finding suggests one of the possible pathways by which gut microbiota can modulate local immune responses in breast tissue.

In the context of tumor eradication, the main effector immune cells are macrophages, natural killer (NK) cells, antigen-presenting cells (APCs) such as DCs and B, T lymphocytes. One of the main groups of effector cells that promote cell-mediated immune response are $CD8^+$ T cells that directly combat cancerous cells, while $CD4^+$ T cells known as T helpers (Th) are classified to different subgroups which can suppress or activate immune response (de la Cruz-Merino et al. 2017). Th1 cells are involved in cell-mediated immunity and are among the body's chief anticancer guards, while Th2 cells promote the body's humoral response, which is the main defense against extrinsic pathogens. The imbalance in Th1:Th2 immune response has been implicated in the etiology of BC. Therefore, favoring the immune system toward a Th1 response seems applicable in BC prevention and treatment (Zamarron and Chen 2011).

On the other hand, regulatory $CD4^+$ $CD25^+$ T cells (Treg) and myeloid-derived suppressive cells (MDSCs) suppress the immune response against cancerous cells and thus contribute to the evasion of tumors from immune surveillance, leading to tumor progression (Zamarron and Chen 2011).

The imbalance between immune suppressive cells and effector cells can hinder the stimulation of adequate immune response which will lead to tumor progression; this can explain why the low ratio of $CD8^+$/Treg is associated with poor prognosis of BC patients (Liu et al. 2011). Some studies shed light on the effect of gut microbiota composition on $CD8^+$/Treg ratio and its role in response to cancer immunotherapy. Studies show that the presence of *Bacteroides thetaiotaomicron* and *Bacteroides fragilis* in the gut microbiota of mice melanoma model was associated with an increased ratio of $CD8^+$/Treg and enhanced the efficacy of immune checkpoint blockade therapy (Vétizou et al. 2015, 2016). Also in metastatic melanoma patients, gut microbiota enriched with the *Faecalibacterium* genus was associated with the downregulation of peripheral blood Tregs and longer overall survival of patients (Chaput et al. 2017). Also, another study in melanoma patients receiving immune checkpoint inhibitors revealed that the presence of Clostridiale*s*, *Ruminococcaceae*, or *Faecalibacterium* was associated with increased $CD8^+$/Treg ratio and enhanced therapeutic outcomes, while the abundance of *Bacteroides* in the gut was associated with increased Tregs and MDSCs and poor prognosis (Gopalakrishnan et al. 2018). These studies highlight the immunomodulatory potential of gut microbiota composition in other types of cancer including BC.

Also, cytokines play an integral role in the regulation of the immune system. Some cytokines are pro-inflammatory (PI) that promote immune response, while others are referred to as anti-inflammatory (AI), which exert immunosuppressive effects. Interleukin-12 (IL-12) is among PI cytokines that can directly activate NK cells and interferon-γ (IFN-γ) secretion, boosting the immune response against BC

(Agaugué et al. 2008). While IL-4, IL-6, Transforming growth factor-β (TGF-β), and Granulocyte-macrophage colony-stimulating factor (GM-CSF) are among AI cytokines, which can activate MDSCs (Gabrilovich and Nagaraj 2009). IL-6 also plays a key role in the regulation of estrogen synthesis in peripheral tissues such as breast tissues and is also associated with the induction of angiogenesis (Purohit et al. 2002; Gopinathan et al. 2015). IL-10 is also among major immunosuppressive cytokines (de la Cruz-Merino et al. 2017). As discussed previously, there is solid evidence regarding the active role of specific gut bacterial strains in the secretion of PI and/or AI cytokines, which can determine the suppression or activation of the immune system. In this sense, the microbiota-dependent immunomodulatory role in animal models of BC and its therapeutic potential in BC patients will be discussed in Sect. 8.4.

8.2.2 Role of Gut Microbiota on Estrogen and Phytoestrogen Levels

High levels of estrogen are associated with increased risk of developing ER^+ BC which is the most common subtype of BC (Pike et al. 1993). Activation of ER by estrogen and ER ligands triggers cell proliferation which is the hallmark of BC (Doisneau-Sixou et al. 2003). There is evidence regarding the regulatory role of gut microbiota in estrogen metabolism and its circulating levels. Certain strains of gut microbiota secrete β-glucuronidase which can de-conjugate estrogen to its active form which binds with ERs (Shapira et al. 2013). Besides, de-conjugation of estrogen enables its reabsorption via the enterohepatic cycle leading to increased estrogen levels. The *Clostridium leptum* cluster and the *Clostridium coccoides* cluster, of the Firmicutes phylum, are the main group of bacteria that possess β-glucuronidase enzymes. The *Escherichia/Shigella* bacterial group, belonging to the Proteobacteria phylum, and also *Streptococcus* bacteria are among β-glucuronidase-producing bacteria (Dabek et al. 2008).

The case–control study by Goedert et al. showed that BC patients had higher urinary estrogen levels and less diverse fecal microbiota composition compared to healthy controls. However, no link was observed between the estrogen levels and microbiota differences. Authors suggested that gut microbiota may affect the risk of BC via estrogen-independent pathways (Goedert et al. 2015). In the following case–control study, the authors suggested that estrogen-related pathways in BC risk were associated with immunoglobulin A $(IgA)^-$ microbiota, while IgA^+ microbiota was associated with immune-mediated pathways (Goedert et al. 2018). IgA is the mucosal secretory immunoglobulin that is produced in response to the enteric pathogens. This antigen-specific antibody can then bind to the pathogens in the intestinal lumen and form an IgA coat on their surface. This process can also happen with commensal bacteria, with less potent forms of IgA. Generally, IgA coating can be a distinguishing sign of disease-driving bacteria and also the degree of the inflammatory response to gut bacterial species (Palm et al. 2014).

It is also noteworthy that the metabolic activity of gut microbiota can lead to the production of certain chemical compounds which can, in turn, alter the microbial

composition and function of the gut, promoting the inflammatory responses which can consequently contribute to tumor initiation (DeLuca et al. 2018).

There is also evidence regarding the active role of gut microbiota in metabolizing the phytoestrogen compounds in our diet to their biologically active metabolites. Isoflavones, ellagitannins, and lignans are main groups of phytoestrogens in our diets which are metabolized to equol, urolithins, and enterolignans, respectively, by gut microbiota (Gaya et al. 2016). Phytoestrogen metabolites have a dual role in the context of BC due to their estrogenic and anti-estrogenic characteristics (Rietjens et al. 2017). The estrogenic effect of phytoestrogens is owing to their ability to bind to ERs. On the other hand, due to the weak binding of phytoestrogens to ERs, they can exert anti-estrogenic effects as a result of preventing the high-affinity estrogen from binding to ERs. This is the underlying reason why phytoestrogen consumption can be associated with both increased and/or decreased risk of ER^+ BC occurrence. Apart from the interaction of phytoestrogens with ERs, they can also contribute to cancer prevention by enhancing the clearance of carcinogens and suppression of inflammation and ROS production as a result of interacting with transcription factors such as nuclear factor-kB (NF-kB) and nuclear factor erythroid 2-related factor 2 (NRF2) (Mocanu et al. 2015). Besides, the metabolic activity of gut microbiota can lead to the production of bioactive compounds that can affect gut microbial composition reciprocally. An example of this can be found in a study, which revealed that soy isoflavones in the diet were converted by gut microbiota to the metabolites that favored the proliferation of *Bifidobacterium* spp., while prohibiting the growth of *Clostridiaceae* (Nakatsu et al. 2014). This prohibition can be beneficial in the context of BC, since the *C. leptum* and *C. coccoides* are among the bacterial clusters with β-glucuronidase enzyme and as mentioned previously can raise the estrogen level and consequently increase the risk of ER^+ BC occurrence. Besides, BC patients were found to have higher levels of *Clostridiaceae,* highlighting the potential role of them in BC etiology (Goedert et al. 2015). On the other hand, *Bifidobacterium* SPP. are reported to have anticancer properties (Wei et al. 2018b) thus boosting their proliferation can be helpful in BC.

Despite the evidence regarding the role of gut microbiota in the metabolism of estrogen and phytoestrogen compounds, more studies are warranted to elucidate the estrogen-mediated role of gut microbiota in the risk of BC.

8.3 Probiotics and Their Mechanisms of Altering the Gut Microbiome

According to the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), probiotics are live microorganisms that confer health benefits upon the host when consumed in adequate quantities (FAO and WHO 2002; Azad et al. 2018). The main probiotic products contain gram-positive bacteria such as *Bifidobacteria* and lactic acid bacteria (LAB) including *Lactobacillus* spp., *Lactococcus* spp., *Enterococcus* spp. and *Streptococcus* spp., etc. (Azad et al. 2018). Other probiotic strains are *Escherichia*, *Propionibacterium*, and some yeast

genera such as *Saccharomyces* (Azad et al. 2018). Nowadays, probiotics are marketed as food products, probiotic supplements, and even drugs (Parvez et al. 2006; Sanz et al. 2008). Food products containing probiotic bacteria are mostly dairy products such as milk and yogurt, which are usually fermented with *Lactobacillus* and *Bifidobacterium* species. The main strains of *Lactobacilli* used in probiotic products include *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus rhamnosus,* and *Lactobacillus helveticus* (Azad et al. 2018). Non-dairy sources of probiotics include beverages, fruits, and vegetable products. Also, non-conventional forms of probiotic products such as chocolate and cereal are being introduced into the market (Awaisheh 2012). There is a plethora of studies about the health benefits of probiotic consumption; also, the preventive and therapeutic roles of probiotics in cancer, allergy, generative and metabolic diseases have been extensively studied during recent years (Kechagia et al. 2013; Nazir et al. 2018).

Probiotics exert their health benefits via different mechanisms including the inhibition of pathogen growth, immunomodulation, triggering the epithelial cell proliferation, and most importantly, regulation of gut microbial composition and function (Thomas and Versalovic 2010). A variety of approaches exist for manipulation of the gut microbiome such as dietary compounds, fecal microbiome transplant (FMT), prebiotics, and probiotics (Sonnenburg and Fischbach 2011; Young 2016). Probiotics are among the most used approaches to manipulate and rebalance the gut microbiota composition due to their appropriate characteristics such as ease of production and safety. Probiotics can manipulate gut microbiota in favor of the beneficial bacteria population growth by secretion of growth substrates. Besides, probiotics can inhibit the growth and function of pathogen bacteria via competition for nutrients and receptor binding sites on the intestinal mucosa, production of inhibitors, antimicrobial and metabolic substances, and regulation of intestinal immunity in response to intestinal microbes (Hemarajata and Versalovic 2013). In this sense, probiotics can help to restore the balance of gut microbial communities in dysbiosis. Some studies have investigated the effect of probiotics on the intestinal microbiota composition and function by deploying different tools such as metagenomics sequencing, elucidating the potentiality of probiotics in altering gut microbiota (Hemarajata and Versalovic 2013). However, the results of the two recent studies raised doubts regarding the efficiency of probiotics in gut microbiota manipulation. Results showed that probiotics colonize in the GI tract of people in a personalized way and some people are resistant to probiotic colonization (Zmora et al. 2018). Besides, the administration of probiotics to counterbalance the antibiotic-induced dysbiosis, even delayed the reconstitution of the gut microbiota composition (Suez et al. 2018).

Conclusively, studies show that probiotics may be capable of stabilizing and tailoring the gut microbiota composition and function; but more studies are warranted to elucidate the association of such manipulation with the desired therapeutic outcomes. Besides, individual differences in response to probiotics should be taken into account before prescribing a regimen based on probiotics.

8.4 Animal Studies

8.4.1 Probiotics Preventive and Curative Role in Animal Models of BC

Here we review the animal studies separately by supplemented species of bacteria and state the dominant mechanism underlying these observed benefits based on the effect of these bacterial strains on immune response in BC. A summary of the mentioned studies can be found in Table 8.1. Details of immune signals in the context of cancer, including BC, were previously discussed in Sect. 8.2.1.

8.4.1.1 *Lactobacillus acidophilus*

Several studies have investigated the effect of supplementation with *L. acidophilus* ATCC 4356 on the mammary tumor-transplanted mice (Soltan Dallal et al. 2010; Maroof et al. 2012; Imani Fooladi et al. 2015). These studies noticed longer survival in bacteria-fed animals, which was exerted through Th1 bias manifested by a decline in IL-4 cytokine, an increase in the production of INF-γ and activity of NK cells.

In another study, the same strain of *L. acidophilus* was orally administered to the mammary tumor-bearing mice (Yazdi et al. 2010). Similarly, the result of splenocyte culture displayed a shift toward the Th1 response, which was revealed by IL-12 increase and downregulation of tumor TGF-β. This shift was confirmed by the evaluation of a delayed-type hypersensitivity response. Besides, the tumor growth rate and tumor volume were significantly lower in the bacteria-treated group.

8.4.1.2 *Lactobacillus reuteri*

Dallal et al. investigated the effect of *L. reuteri* ATCC 23272 ingestion on mice breast adenocarcinoma (Dallal et al. 2012). The NK cell activity and tumor-specific lymphocyte proliferation were significantly higher in the case group demonstrating a boost in immune system after probiotic ingestion. In another study, the effect of a different *L. reuteri* strain (ATCC-PTA-6475) was evaluated in two different mice models with a predisposition toward developing breast cancer: a westernized diet model and a genetically susceptible model (Lakritz et al. 2014). Oral administration of the probiotics was able to inhibit both animal models from the progression of tumor and significantly increased the tumor-free survival. The observed protection that was transplantable to the mammary tumor animal model was exerted through the upregulation of apoptosis and $CD4^+$-$CD25^+$ regulatory T cells.

8.4.1.3 *Lactobacillus casei*

The effect of milk fermented with *L. casei* CRL 431 on mice breast cancer was investigated in two studies by Aragon and colleagues (Aragón et al. 2014, 2015). In a study, they showed the preventive effect of this probiotic through inhibiting or delaying tumor growth alongside its immunomodulatory effect when administered after tumor detection. The evaluation of cytokine profile revealed a reduction in IL-6 and an increase in IL-10 concentration. In another study, they showed suppressed tumor growth, angiogenesis, and lung metastasis following administration of this

Table 8.1 Summary of studies on the administration of probiotics in animal BC model

Strain	Effect	Mechanism	Reference
L. acidophilus	↓ tumor growth	↑ IL-12	Yazdi et al. (2010)
	↓ tumor growth	↑ Th1	Maroof et al. (2012)
	↑ immune response	↑ Th1 ↑ IFN-γ ↓ IL-4 and IL-10	Imani Fooladi et al. (2015)
	↑ immune response ↑ survival	↑ IFN-γ ↓ IL-4	Soltan Dallal et al. (2010)
L. reuteri	↑ mouse survival	↑ NK cells ↑ tumor-specific lymphocyte	Dallal et al. (2012)
	↑ immune response ↑ apoptosis	Microbial activation of lymphocytes	Lakritz et al. (2014)
L. casei	↓ tumor growth ↓ angiogenesis ↓ metastasis	↑ $CD8^+$ and $CD4^+$ T cells	Aragón et al. (2014)
	↓ tumor growth	↑ IL-12 ↑IFN-γ ↑ NK cells	Aragón et al. (2015)
	↓ tumor growth ↓ angiogenesis	↓ IL-6	Soltan Dallal et al. (2012)
	↓ tumor growth ↓ angiogenesis	Activation of neutrophils and monocytes	Kaga et al. (2013)
L. helveticus	↓ tumor growth	↓ IL-6 ↑ IL-10 ↑ IgA ↑ $CD4^+$ cells	de Moreno de LeBlanc et al. (2005a, b)
	↓ tumor growth	↓ IL-6 ↑ IL-10 Induction of apoptosis	de Moreno de LeBlanc et al. (2005a, b)
	↓ tumor growth	↓ IL-6 Induction of apoptosis	Rachid et al. (2006)
L. plantarum	Inhibition of tumor frequency	↑ $CD4^+$ cells ↑ TNF-α	Kassayová et al. (2014)
	↑ mouse lifespan	↑ IFN-γ ↑ TNF-α ↑ IL-12 ↑ NK cells activity	Yazdi et al. (2012)

IL-interleukin, *Th*-T helper, *IFN*-interferon, *NK*-natural killer, *Ig*-immunoglobulin, *TNF*-tumor necrosis factor

probiotic that were related to the regulation of immune response through reduction of macrophage infiltration and upregulation of $CD8^+$ and $CD4^+$ lymphocytes. Soltan Dallal et al. conducted a study to evaluate the effect of *L. casei ssp. casei* ATCC 39392 against invasive duct carcinoma (Soltan Dallal et al. 2012). The results indicated a decrease in the rate of tumor growth and a prolonged survival exerted through the production of IL-12 and IFN-γ, implicating Th1 shift. Another study by

Kaga et al. aimed to elucidate the effect of *L. casei shirota* in combination with soymilk on chemically induced BC (Kaga et al. 2013). The results were promising as the combination was able to slow the tumor growth and inhibit tumor vascularization.

8.4.1.4 *Lactobacillus helveticus*

In an attempt to investigate the effect of milk fermented by *L. helveticus* on mammary tumors, a group of scientists conducted three studies. They evaluated the immune response after the administration of milk fermented by the two mentioned strains of *L. helveticus* in normal and BC model (de Moreno de LeBlanc et al. 2005a, b). Similar to their first work, IL-6 decrease and IL-10 increase were more pronounced in *L. helveticus* R389 group. In this group, the presence of tumor was associated with the increase in $CD4^+/CD8^+$ ratio due to the boosted amplification of $CD4^+$ cells. Furthermore, in both strains apoptosis was heightened due to the downregulation of cell survival protein, Bcl-2. In another study, the effects of supplementation of milk fermented by *L. helveticus* R389 strain on mammary tumors were evaluated by following various tumor and immune factors for two months. The delay in tumor growth in animals fed with fermented milk was in agreement with the reduction in IL-6, Tumor Necrosis Factor (TNF)-α, and IFN-γ (de Moreno de LeBlanc et al. 2005a, b).

In the last study, mice hormone-dependent BC model was orally administered with milk fermented by *L. helveticus* R389 or L89 (proteolytic-deficient variant) (Rachid et al. 2006). Both *L. helveticus* strains hindered tumor development through a decrease in IL-6. Further investigation with R389, related the immunomodulatory effect of this strain to the potentially released peptides in R389 fermented milk which may increase the production of IL-10 and apoptotic cells.

8.4.1.5 *Lactobacillus plantarum*

Kassayova et al. carried out a study to test the effect of *L. plantarum* LS/07 and oligofructose-enriched inulin combination on chemically induced mammary tumors in rats (Kassayová et al. 2014). The combination not only limited the incidence of tumors but it also reduced the size of tumors. In another study, the effect of *L. plantarum* ATCC 8014 probiotic with or without selenium nanoparticles (SeNP) was studied in the BC model (Yazdi et al. 2012). The combination increased survival and decreased tumor volume through Th1 immune response bias manifested by elevated IL-12, IL-2, and TNF-α.

8.4.2 Shortcomings of Animal Studies

Murine models have extensively been used in biomedical research including gut microbiota-related studies and have provided valuable insights into the role of gut microbiota in pathological conditions such as cancer. Despite the numerous similarities between the GI tract of mice and humans, some differences can affect the interpretation of gut microbiota-related studies. First, since the considerable amount of the mouse diet is based on the indigestible food, the colon and cecum

capacity in mouse have been expanded to enable the nutrient extraction from its diet (Treuting and Dintzis 2012). Mouse cecum is its main site for fermentation, while fermentation in human is restricted to the colon and is not observed in the vestigial cecum. The mentioned difference in the fermentation site between mice and humans can affect the composition of microbiota in the colon. Furthermore, appendix, an organ in humans which has been suggested to have a role in the reconstitution of the gut microbiota after disturbances, owing to its ability to store beneficial bacteria, is absent in the mice (Smith et al. 2017). Apart from the macroscopic anatomical differences, the microscopic structures of the human and mouse gastrointestinal (GI) tract also have differences, which might affect the GI microbiota composition by creating different ecological micro-niches. In addition, the differences at the cellular level including the diversity of the distribution of goblet and paneth cells between mouse and human might affect the microbiota composition due to the immunomodulatory roles of these cells (Nguyen et al. 2015). Besides the anatomical differences, studies also show the differences between gut microbiota composition between humans and mice. In addition to the mentioned intrinsic anatomical and compositional differences between mouse and human GI, also other pitfalls in exploiting mouse models in gut microbiota-related studies exist. In humans, real-life gut microbiota is shaped by various factors such as genetic and environmental backgrounds including diet, medical history and even the mode of birth or feeding. These factors are absent in mice, lowering the accuracy of mice gut microbiota-related models. Besides, the inbred mouse strains cannot reflect the genetic variations in the human population, which impose differences in gut microbiota composition between individuals, due to the genetic homogeneity of these strains.

Beyond the concerns mentioned above regarding the fact that the healthy mouse GI tract cannot fully represent healthy human GI, the complexity becomes even more when the study is conducted in animal disease models. For instance, in a mouse model of BC, apart from the intrinsic shortcomings associated with the mouse BC model, one cannot be sure whether the dysbiosis condition associated with BC observed in human individuals will be the same in the mouse model. This becomes of great importance when the study aims to target the gut microbial imbalance in BC as the therapeutic intervention, which is the case in probiotic administration. Besides, hesitations exist regarding the differences in the ability of probiotics to alter gut microbiota in mice or humans. The study of Zmora et al. showed that the murine gut microbiome prevented the colonization of the human-targeted probiotics, while results in human were person-specific (Zmora et al. 2018). In the end, the host-gut microbiota interactions are host-specific and translation of the observations of gut microbiota cross-talk with the immune system in animal models to clinical outcomes should be done with caution.

8.5 Human Studies

As mentioned earlier, *in vitro* and *in vivo* studies have displayed the promising capability of certain probiotics in prevention and treatment of BC. Nonetheless, there is a paucity of human and clinical research on the role of probiotics in BC, with no study evaluating its therapeutic role in BC patients and with the available studies mostly examining the effect of dairy products.

In a case–control study in Paris, 1010 BC patients and 1950 controls who were awaiting a non-malignant-related operation were surveyed about the consumption of milk products and alcohol (Lê et al. 1986). This study found out a significant inverse association between consumption of yogurt, which is a fermented dairy product, with BC incidence. Although, this impact was not strong enough to reverse the positive correlation between alcohol intake and BC risk. In 1989, another case–control study was conducted in the Netherlands to evaluate the association between dairy product consumption and the development of BC (Veer van't et al. 1989). The study consisted of 133 BC patients and 289 healthy controls that were grouped in two age categories of 25–44 and 55–64 years old. This study found out that the consumption of fermented dairy products in both age categories was significantly lower in the patients group compared to the controls. In 1991, the results of this study were analyzed to determine the dietary regimen with the lowest risk of BC development (Veer van't et al. 1991). This study found out that a diet that consists of fermented dairy products with low fat, and high fiber is significantly associated with protection against BC. The observed preventive effect of fermented milk products is possibly related to the impact of LAB on the enterohepatic cycle of estrogen along with its regulatory effects on the immune system. Besides, it was evidenced that three LAB enzymes (β-glucuronidase, nitroreductase, and azoreductase) exist in fermented dairy products that can prevent procarcinogens from becoming carcinogens.

In a more recent population-based case–control study in Japan, 306 BC patients and 662 healthy controls of 40–55 years old were interviewed to assess the association of BC and several risk factors including diet and lifestyle (Toi et al. 2013). It was observed that the consumption of beverages containing *L. casei shirota* (LcS) (equal or more than four times a week versus less than four times a week) since adolescence was negatively associated with the risk of BC incidence. This preventive effect may lie behind the ability of LcS to activate NK cells and promote an immune response.

Human studies evaluating the effect of probiotics on BC, especially their therapeutic capacity are limited. This may be due to the uncertainties regarding the effects of different strains and doses of probiotics on BC and their possible interaction with patients' standard therapeutic regimens. However, to translate our bench knowledge to the bedside understandings and advance the available therapeutic regimens, it is warranted to conduct well-designed clinical trials to examine the probiotics genuine capacities in the treatment of BC.

8.6 Alleviating Role of Probiotics in Chemotherapy-Induced Side Effects

BC can be treated by surgery, chemotherapy, hormone therapy, radiotherapy, and targeted therapy. Alongside the therapeutic benefits of these treatments, they can cause several side effects. In particular, chemotherapy can damage the GI mucosa and cause side effects such as diarrhea and mucositis.

The plausible ability of probiotics to alleviate the side effects of cancer treatment through different mechanisms such as repairing the intestinal barrier and restoring the balance in gut microbiota have been assessed in several studies. Here, studies in the setting of BC are elaborated.

El-Atti et al. reported a stage IV BC case who was suffering from grade 2 diarrhoea following chemotherapy with lapatinib and capecitabine (Abd El-Atti et al. 2009). Initially, the diarrhoea was managed by loperamide but the gradual increase in the abdominal pain and bloating caused the drug's discontinuation. Following the cessation of loperamide and the subsequent deterioration of diarrhoea to grade 3, the patient was prescribed with a multispecies combination of probiotics (450 billion bacteria from 8 LAB strains) two times a day. The probiotics immediately took effect and mitigated the frequency and severity of diarrhoea to normal levels. It was stated that diarrhoea came back immediately every time the combination was stopped. This study suggests the potential benefits of probiotics in treating chemotherapy-induced diarrhoea.

Miller et al., proposed possible mechanisms by which probiotics can exert their protective effects on reducing chemotherapy-induced diarrhoea. First, probiotics can rebalance the gut microbial composition and prevent attachment of pathogenic bacteria to gut lumen by providing a physical barrier. Second, LAB strains may lower the pH of the intestinal mucosa, which can downregulate the growth of pathogenic bacteria, and last, probiotic strains may be able to metabolize carcinogenic agents into harmless products (Miller and Elamin 2009).

Genitourinary syndrome of menopause is a common side effect of chemotherapy and hormone therapy in BC patients. A randomized placebo-controlled double-blinded pilot study investigated the effect of oral supplementation with a combination of 4 LAB strains on vaginal atrophy and vaginal microbiota in 22 BC patients (Marschalek et al. 2017). The results were promising as the probiotics were able to improve the patients' Nugent score toward normal while the Nugent score in the control group was significantly deteriorated. This study underscores the ability of probiotics in improving vaginal microbiota in BC patients receiving Chemotherapy. It also signifies the effectiveness of oral formulation as a more patient-friendly option for this indication.

In another study, Tooley et al. aimed to investigate the effect of a promising strain of bacteria on methotrexate (MTX)-induced mucositis in mammary tumor bearing rats (Tooley et al. 2006). This strain, *Streptococcus thermophiles* (TH-4), had been able to improve the MTX-induced mucositis in healthy rats. However, in contrast to this study TH-4 was not effective against the MTX-induced mucositis in tumor-bearing rats (Tooley et al. 2011). To conclude, despite some promising results there

is much more to know about the real potential of probiotics in the setting of BC chemotherapeutic-induced side effects.

8.7 Safety Concerns of Probiotics in General and in Breast Cancer

The use of probiotics in BC and their efficacy have been discussed in previous parts. However, there are some concerns as to whether or not probiotics can do more harm than good. In this section, we discuss these concerns, their basis and relevance, and related clinical evidence.

Numerous studies and clinical trials have been conducted to investigate probiotics' efficacy, and safety to a lesser extent, for treatment and prophylaxis of GI diseases. In the course of these studies, several concerns regarding the safety of probiotics have arisen. The main concerns include the possibility of gene transfer, risk of transmigration and systemic infections, and unwanted metabolic activities.

8.7.1 Gene Transfer

The first concern is that probiotics can transfer their genes to other bacteria and possible pathogens in the GI tract, a process called horizontal gene transfer (HGT), and through this, add to the gene pool of the gut microbes and cause antimicrobial resistance (The EFSA Panel on Additives and Products or Substances used in Animal (FEEDAP) 2018). Indeed, LAB, the most commonly used bacteria in the probiotic products, are resistant to several antibiotics, the most common being tetracycline, erythromycin, chloramphenicol, and vancomycin (Álvarez-Cisneros 2019). Vancomycin resistance, for example, is due to an inherent difference in some of the LAB cell wall structure encoded by their chromosome which is not transferable, while tetracycline resistance is conferred by genes such as tet (M) located on a potentially transferable plasmid (Wright 2007; Ammor et al. 2008; Gueimonde et al. 2013). Indeed, HGT has been established in various species of *Lactobacilli* both *in vitro* and *in vivo*, conducted mostly on rodents (Schjørring and Krogfelt 2011; Lerner et al. 2019). However, HGT does not happen in all bacteria, and there is limited evidence substantiating HGT in the human intestine. Moreover, it is unknown whether the genes, if transferred, will be incorporated in the genome of the bacteria (Brooks et al. 2016). To date, only 1 study has been performed on humans in this regard, where transferability of tetracycline resistance gene tet(W) was established from *L. reuteri* to *Enterococci*, *Bifidobacteria*, and *Lactobacilli* based on samples from human feces (Egervarn et al. 2010).

One hypothesis may be that resistance genes not on mobile genetic elements (such as plasmids) could be beneficial in some cases. For instance, when an antibiotic for a sensitive pathogen is administered, if probiotic bacteria are resistant to this antibiotic, they may have better efficacy in restoring the gut microbiome and prevent diarrhoea after antibiotic administration. Also, since the genetic material coding for

the resistance is not mobile, the probiotic cannot confer it to the pathogens (Gueimonde et al. 2013; Cohen 2018).

Nonetheless, for the pharmaceutical and food industry, it is best to use species whose genomes are studied more with regards to the resistance genes until further research is conducted. These species include *Bifidobacterium adolescentis*, *Bifidobacterium animalis*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium longum*, *L. acidophilus*, *L. casei*, *Lactobacillus fermentum*, *Lactobacillus gasseri*, *Lactobacillus johnsonii, L. reuteri, Lactobacillus paracasei*, *L. plantarum*, *L. rhamnosus*, and *Lactobacillus salivarius* (de Simone 2019). From the regulatory standpoint of European countries and Canada, antibiotic-resistant genes encoding clinically important antimicrobials on mobile gene elements must be absent (Wassenaar and Klein 2008; The EFSA Panel on Additives and Products or Substances used in Animal (FEEDAP) 2018).

8.7.2 Transmigration and Systemic Infection

Transmigration is a process in which microorganisms such as bacteria and their products such as toxins and fragments cross the gut epithelium and possibly reach the systemic circulation. This often occurs in special populations, such as immunocompromised, trauma, or postoperative patients (Wells and Erlandsen 1996).

Two factors that have been hypothesized to cause this transmigration are adhesion to the gut cells and change in permeability of the gut wall by probiotics. Adhesion, one of the primary criteria when searching for beneficial probiotics, helps them bind to gut cells, and this, in turn, allows them to populate and exert their benefits, and to function as a natural barrier against pathogens (Collado et al. 2007; Li et al. 2015).

While studies conducted in animals indicate that probiotics lower the transmigration of other bacteria and enhance gut barrier integrity, case reports exist for bacteremia with *Lactobacilli* or *Bifidobacteria* or fungemia with *Saccharomyces* (Rao and Samak 2013; Singhi and Kumar 2016). In many cases, the isolated strain could not be traced back to the ingested probiotic strain, or prior intentional probiotic use was not reported. Also, in cases where the strains were indistinguishable from the administered probiotic, patients were among special populations or critically ill and receiving prophylactic treatment for diseases that otherwise would have been fatal (Sipsas et al. 2002; Hempel et al. 2011). Indeed, LAB in immunocompromised and critically ill patients have been administered in clinical trials without harm (Jain et al. 2004; Rayes et al. 2005; Zhang et al. 2013; Gorshein et al. 2017; Sadanand et al. 2019).

In light of these case reports and after consumption of probiotics containing *L. rhamnosus* GG increased rapidly in Finland, the number of *Lactobacillus*-associated bacteremia in healthy people was assessed in an epidemiological study conducted between 1990 and 2000 (Salminen et al. 2002). *Lactobacilli* was positive in 0.02% of all blood cultures obtained, but no trends indicated that the increase in the use of *L. rhamnosus* GG as a probiotic was related to the increase in

Lactobacillus bacteremia. Among the blood cultures positive for bacteria, *Lactobacilli* were present in 0.24%, which was in line with what had been reported before. Also, adhesiveness of eight strains isolated from positive cultures was studied *in vitro*, and it was concluded that adhesiveness does not seem to be a prerequisite for systemic infection (Kirjavainen et al. 1999).

In a more recent study, the incidence of *Lactobacillus* bacteremia in Stockholm was investigated between 1998 and 2004 (Sullivan and Erik Nord 2006). Among 53 cases available for examination, 32 of the cases were *Lactobacilli*, and half of the cases were, in fact, polymicrobial. The authors reported that during this period, the incidence rate of *Lactobacilli*-induced bacteremia was not changed.

Taken together, it appears that probiotics are safe in healthy individuals, and there is evidence of safe use for the treatment and prevention of diseases in critically ill populations and immunocompromised patients. Nevertheless, the pros and cons of probiotic administration should be carefully weighed in the immunocompromised and critically ill patients.

8.7.3 Unwanted Metabolic Activities

Another risk associated with probiotics is lactic acidosis due to the accumulation of D-lactate as a metabolic byproduct of some of the *Lactobacillus* and *Bifidobacterium* species (Lerner et al. 2019). Human metabolism produces L-lactate, but it is not apt for excretion of D-lactate. Thus, the use of D-lactate producing probiotics is discouraged or must be assessed carefully, especially in patients with short bowel syndrome, those who have undergone bypass surgery, infants whose renal function is not matured yet, and critically ill patients such as end-stage cancer patients with high systemic lactic acid load (Sanders et al. 2010; Reid et al. 2017; van den Nieuwboer and Claassen 2019). The outcome of a trial named PROPATRIA, in which the efficacy of strains of *Lactobacilli* was assessed in acute pancreatitis, is evidence for this caution. Although the study had design flaws, it reported a higher mortality rate in the probiotic arm and raised concerns about the safety of probiotics. This unforeseen outcome is attributed to high acid concentrations, including D-lactate, as a result of the high carbohydrate fed to the patients through an enteral tube. This might have caused the subsequent intestinal ischemia and further complications (van den Nieuwboer and Claassen 2019).

Another concern regarding unwanted metabolic activities is the theoretical risk of bile acid de-conjugation. LAB can de-conjugate bile acid using their Bile Salt Hydrolase (BSH), which produces free bile acid. This process is important for LAB survival in the gut and is another selection criteria for probiotics. However, the free bile acid is open to modification to secondary bile acids such as deoxycholic acid and lithocholic acid. Since these acids can accumulate and enter the enterohepatic circulation, it has been hypothesized that they could play a role in some GI diseases such as colon cancer and gall bladder (Pavlović et al. 2012). However, it has been shown that *Lactobacilli* and *Bifidobacteria* are not able to

modify the free bile acid into secondary acids, and there is no clinical evidence to substantiate this theory as well (Snydman 2008).

8.7.4 Adverse Effects in Practice

There is a myriad of clinical trials and subsequent systematic review articles investigating the efficacy and safety of probiotic products, and these were mostly conducted on GI disorders to treat or prevent diarrhoea in adults, children, or infants (Hempel et al. 2012; Parker et al. 2018).

In terms of safety, the results of randomized clinical trials (RCTs) and case reports differ. Adverse events (AEs) that were attributed to probiotic use, were reported by two trials and included GI disturbances, specifically bloating and flatulence, nausea and vomiting, epigastric pain, and constipation (Parker et al. 2018; Guo et al. 2019). One trial reported significantly more adverse events, namely an increase in thirst and constipation, in the *Saccharomyces boulardii* arm compared to placebo (Guo et al. 2019). Serious AEs in the critically ill or extremely immunocompromised children with risk factors such as central venous device use and conditions associated with bacterial and fungal transmigration have occurred in several observational studies but not RCTs (Guo et al. 2019). In light of the case reports of bacteremia and fungemia with a possible association to probiotics, a 2011 Systematic review of over 194 parallel RCTs conducted by the Agency for Healthcare Research and Quality reported that the difference in the number of AEs between intervention (probiotic treatment) and control arm of the studies was not statistically significant. Also, none of the studies reported probiotic-associated infection (Hempel et al. 2011).

These results have to be interpreted with caution as there are several caveats in the conducted trials (Parker et al. 2018). Different probiotic strains have a different clinical effect and/or risk of AEs, yet not all trials report specific strains that were used (Sanders 2008; Allen et al. 2010; Hempel et al. 2011). Moreover, most studies do not reliably validate the potency and viability of the probiotics used (Suez et al. 2019). For example, out of 163 clinical trials that 14 Cochrane Reviews analyzed, 63% did not specify strain. There is also the issue of under-reporting of AEs and also lack of clear documentation as to what AEs were monitored for. For example, while 73% of the 163 trials mentioned previously, specifically reported side effects, numerous trials only mentioned AEs in passing, citing that the probiotic was "well-tolerated" or using similar statements. It is therefore recommended that investigators report harms as well as benefits of intervention by using CONSORT guidelines, for the gathered data to be homogenous across all studies, and also adhere to Common Terminology Criteria for Adverse Events (CTCAE) system for categorizing the occurred AEs (Hempel et al. 2011).

Furthermore, the majority of gathered data to date is on the *Lactobacilli* alone or in combination with other LAB (mostly *Bifidobacteria*), not on microbes of other commonly used genera such as *Bacillus* and *Enterococcus*. Finally, there is not much evidence regarding the long-term effects of probiotics, and data in the elderly

and pregnant and lactating women are warranted to be compared to other populations since many trials excluded these subgroups.

Reported adverse events in studies investigating the efficacy and safety of probiotics in cancer have the same limitation as discussed previously. A systematic review assessed the safety of probiotic use in people with cancer and examined 12 studies and 7 case reports that met its inclusion criteria. There were reports of death, but none were attributed to probiotic use by the original authors. In five case reports, bacteremia and fungemia, related to probiotic use, were observed in immunocompromised cancer patients (Redman et al. 2014).

Another systematic review evaluating the efficacy and safety of probiotics in the prevention or treatment of chemotherapy- or radiotherapy-related diarrhoea found no difference concerning AEs. Also, no death occurrence was mentioned but for one patient who died of myocardial infarction after three radiotherapy sessions (Wei et al. 2018a).

Data about probiotic use in breast cancer as treatment, prophylaxis, or symptom alleviation is even more scarce, and studies are mostly conducted *in vitro* or on animal models (Ranjbar et al. 2019). Clearly, probiotic use in cancer is still an emerging area.

Altogether, limited data is available for probiotics safety in general and in breast cancer, and there are shortcomings in the conducted studies making interpretation of the results difficult. Available evidence suggests that probiotic use is safe in healthy individuals and most patients, but their benefits should be carefully weighed against their potential risks in infants, and critically ill and immunocompromised patients.

8.8 Regulatory Concerns in Probiotics

In addition to the definition described in Sect. 8.3, a probiotic product must also have the following characteristics (FAO and WHO 2002):

- must be alive when administered,
- must have undergone a controlled evaluation to document health benefits in the target host,
- must be a taxonomically defined microbe or combination of microbes (genus, species, and strain level),
- must be safe for its intended use.

This definition was deemed sufficient by the International Scientific Association for Probiotics and Prebiotics (ISAPP) in 2013 (Hill et al. 2014). In reality, however, regulation frameworks differ among various regions of the globe. Regulations need to address a product's efficacy, safety, manufacturing quality, and validity of health claims (de Simone 2019). Here we discuss the extent to which the USA, European countries, and Canada address these aspects.

In the USA, a probiotic can fall under one of these four categories based on its intended use: a dietary supplement (the majority of the products), food or food ingredient, medical food, or drug or biologic drug (Degnan 2008; de Simone 2019).

Dietary supplements are products which, most importantly, supplement the diet with ingredients such as vitamins, are intended for ingestion, and are not part of a normal diet (The Office of Dietary Supplements 1994). Dietary supplements require Current Good Manufacturing Practice (cGMP) adherence but do not generally require testing quality, efficacy, or premarket approval unless they contain a new food ingredient (Food and Drug Administration 1997; de Simone 2019). Also, manufacturers cannot make disease-specific health claims such as curing or preventing a disease, only general claims regarding functional or structural changes with adjectives such as "regulate," "promote," etc. (Food and Drug Administration 2002). The manufacturer also has to have scientific evidence, available at the discretion of Food and Drug Administration (FDA), that the claims are not misleading and are truthful, and the product also has to be accompanied by a specific statement, explaining that the product has not been evaluated by the FDA (Degnan 2008).

A probiotic can also be categorized under a food additive or food ingredient. Any substance that is "reasonably expected to become a component of food" is a food additive and requires FDA premarket sanction, unless it can be granted Generally Recognized as Safe (GRAS) status. Substances with GRAS status such as the majority of the probiotic bacteria are only subject to post-market checks, and regulations of claims discussed above also apply here (Fijan 2014).

Another category that a probiotic product can be categorized under in the US market is medical food. These are food intended to be administered via an enteral route under the supervision of a physician. Similar to food and dietary supplements, medical food do not need premarket approval if the ingredients are GRAS. Medical food and food category require cGMP as well (Food and Drug Administration 2016).

Finally, a probiotic product can be categorized as a drug and simultaneously a biologic product (Food and Drug Administration 2006; Degnan 2008). In this case, it must acquire premarket approval and comply with the Investigational New Drug (IND) application requirements unless it acquires GRAS status and another status named Generally Recognized as Effective (GRAE) (Food and Drug Administration 2017). To be eligible for GRAS and GRAE, there should be clinical trials up to par with studies conducted for the New Drug Application (NDA) process in terms of quality and number. These studies must establish that the product is safe and effective, and must be published in scientific journals. Finally, experts must reach a consensus that based on these published studies, the product is safe and effective for its intended uses.

Foods and food supplements in Europe are overseen by the European Food Safety Authority (EFSA) which is stricter than the USA and Canada (Pen & Tec Consulting 2018). In Europe, a probiotic can be defined as a food or a food supplement, and in both cases, it falls under the General Food Law (Commission of the European Communities 2008). As probiotics are live microorganisms, they are considered

biological agents. Thus, unless they acquire a qualified presumption of safety (QPS) status, they have to undergo a full safety assessment. QPS status is similar to GRAS status in the USA except it is only for microorganisms, not other additives (Laulund et al. 2017). According to EFSA, the following criteria are required for a microorganism to be granted QPS status (European Food Standards Authority 2013):

- Its taxonomic identity must be well defined;
- The available body of knowledge must be sufficient to establish its safety;
- The lack of pathogenic properties must be established and substantiated;
- Its intended use must be clearly described.

When a microorganism is granted QPS status, it will be included in the regularly updated QPS list. Currently, probiotic species such as *Lactobacillus* spp., *Lactococcus lactis*, and *Bifidobacterium* spp. have QPS status, to name but a few. Microorganisms with safety risks or inconclusive evidence will not be added to the list (European Food Standards Authority 2013). Concerning quality, the products must adhere to GMP and Hazard Analysis of Critical Control Points (HACCP) standards (Laulund et al. 2017).

In the European Union, probiotics must comply with Nutrition & Health Claims Regulation 1924/2006 (NHCR) for their health claims, which are evaluated by EFSA. Currently, all but a handful of health claims have received approval from EFSA (Donovan et al. 2012; Winclove Probiotics 2017). Moreover, as per 2007 European Commission (EC) Guidance on the adaptation of the European Union, Regulation (EC) No 1924/2006, the term "contains probiotic" on the labeling is also considered a health claim since it implies a health benefit per the FAO/WHO definition (International Probiotics Association n.d.). As such, manufacturers and EFSA appear to be at an impasse about health claims in Europe.

In Canada, probiotics are categorized as natural products or as ingredients in food (Health Canada 2019). If probiotics are not intended to be used in food, they are defined as natural products or medicinal ingredients and Natural and Non-prescription Health Products Directorate (NNHPD) is the responsible body for their regulation. For natural products, Canada has defined one regularly updated monograph, which, most importantly, includes the names of accepted strains and their source, dosage forms, quantities of the microbes, storage conditions, and some specifications. Specifications include a demonstration of survivability in the human gut, genotyping and phenotyping requirements, and absence of virulence of each microorganism. For the absence of virulence criteria, the probiotic must have an antibiotic resistance profile, lack toxigenic activity, be susceptible to at least two commercially available antibiotics, and not be able to transfer genes laterally. The quality of the finished product must be based on the Quality of Natural Health Products Guide. There are strain-specific claims that can be labeled for three strains, and other defined claims for all other microorganisms. For example, for *L. rhamnosus* GG, it can be claimed that it "helps to manage acute infectious diarrhoea." For all other microorganisms, either of these two statements can be mentioned: "helps support intestinal/GI health" or "could promote a favorable gut

flora." Canada's approach to probiotic supplements is more streamlined as there is one single monograph for probiotics, and all accepted claims and scientific references are listed.

Probiotics used in food are assessed by the Canadian Food Inspection Agency, which works under the regulations set by Health Canada and must adhere to the Food and Drug Act (Health Canada 2009). Two types of claims can be made for food with probiotics. One is a strain-specific claim which refers to particular benefits of a specific strain of microorganisms, and the manufacturer must have the necessary evidence to support this; to date, no such claims have been accepted by Health Canada (Canadian Food Inspection Agency 2015). Another claim is a non-strain-specific claim which a manufacturer can make if their product contains one or more bacteria from a defined list. The list contains many species of the *Lactobacillus* and *Bifidobacterium* genera. The claims, too, can be made from a defined list without the need for scientific substantiation. For instance, "probiotic that naturally forms part of the gut flora" is one of such accepted claims. If a company wishes to make a new claim not stated above, they must provide scientific evidence and not make vague claims, as exemplified in detail in the Canadian Food Inspection Agency's website (Canadian Food Inspection Agency 2015, 2019).

8.9 Market Trend in Probiotics

The market of probiotics has been rapidly growing and is forecasted to do so in the following years. In 2007, the global market for probiotic ingredients, supplements, and foods was estimated to worth $14.9 billion, and it has currently been estimated to double from $37.7 billion in 2016 to $71.9 billion by 2025, a compound annual growth of 7.49% (Granato et al. 2010; Ahuja and Deb 2018). The Asian Pacific region is anticipated to have the largest market share by 2026 (Inkwood Research 2017). Growing consumer interest for natural products and prevention of health issues through diet, alongside increased disposable income, have been attributed as some of the contributing factors for this growth (Hajela et al. 2010).

8.10 Prospects of Probiotic Administration in the Prevention and Treatment of BC

Mechanisms of preventive, curative, and palliative roles of probiotics in BC are summarized in Fig. 8.1. As has been discussed in this chapter, one of the main roles of probiotics in BC is attributed to their regulation of gut microbiota composition and function. Gut microbiota is associated with BC via estrogen and immune-mediated pathways. However, there is not enough evidence regarding the estrogen-mediated role of gut microbiota in BC as was discussed previously (Goedert et al. 2015). Additionally, the role of gut microbiota in metabolizing phytoestrogens also may exert undesirable outcomes due to the possible estrogenic effects of these compounds. The immunomodulatory role of probiotics in BC treatment is merely

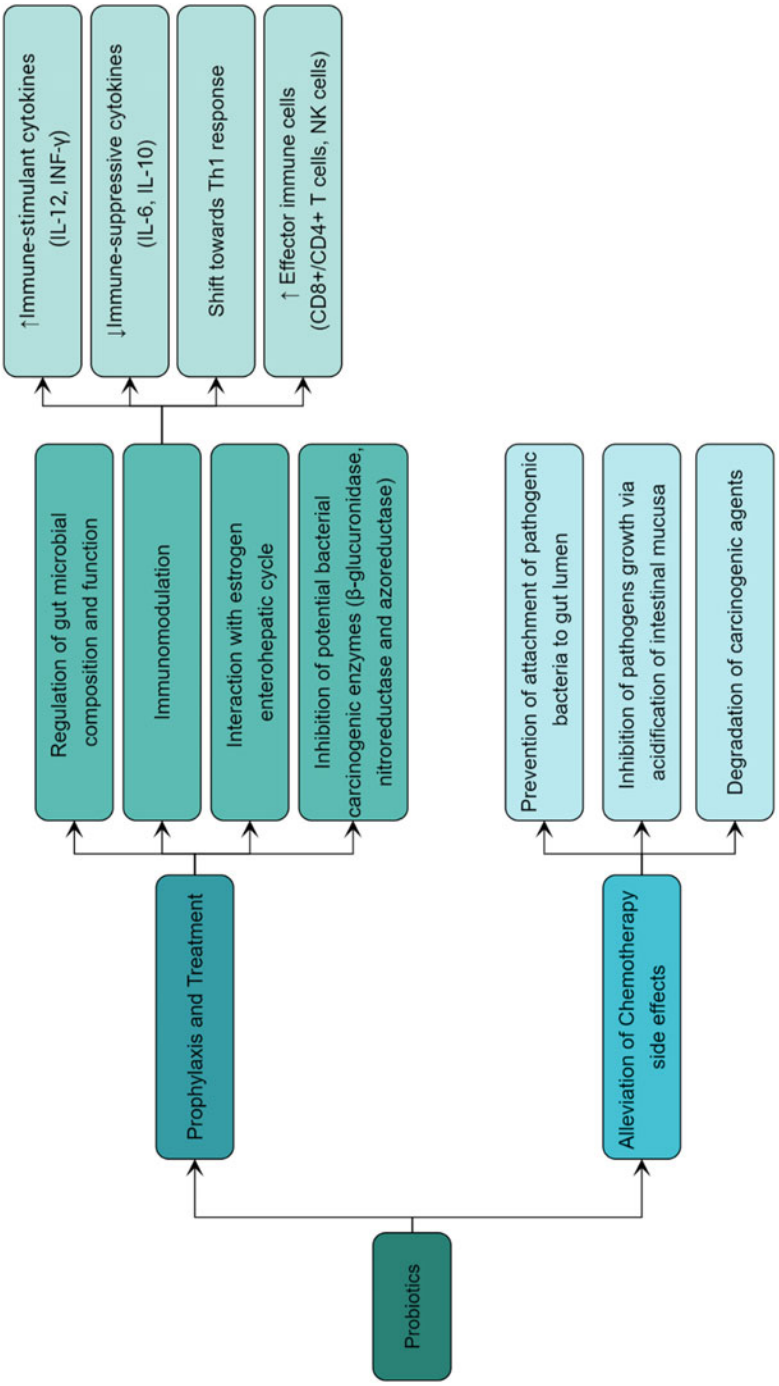

Fig. 8.1 Mechanisms of preventive, curative, and palliative roles of probiotics in breast cancer. *IL* interleukin, *IFN* interferon, *Th* T helper, *NK* natural killer

Table 8.2 Summary of efficacy vs concern of probiotics in BC

Efficacy	Concern
• Preventive role: Consumption of LAB-containing FMPs, LcS, and yogurt: ↓ Risk of BC in case–control studies • Curative role: Consumption of LAB in animal studies: ↓ Tumor growth Via Immunomodulation • Palliative role: 1. ↓ Chemotherapy-induced diarrheain BC patients 2. ↑ Nugent score in BC patients receiving chemotherapy	• Under-reporting AEs in clinical studies or using general terms • Lack of sufficient evidence for long-term use • Lack of enough evidence in the elderly and pregnant and lactating women • General safety concerns of probiotics owing to their gene transferability, transmigration, and systemic infection, and unwanted metabolic activities • The personalized response of patients to probiotic colonization • Risk of over/or under-treatment in palliative or adjuvant therapy owing to the metabolic activity of probiotics

LAB lactic acid bacteria, *FMPs* fermented milk products, *LcS* Lactobacillus casei shirota, *AE* adverse effect

based on the observations in animal studies, which cannot fully predict clinical outcomes but can provide helpful insights, as discussed in Sect. 8.4.2. On the other hand, it is not realistic and ethical to design clinical trials in humans with probiotics as the only therapeutic intervention to evaluate their curative role in BC patients. Probiotic administration as an adjuvant to the main therapies including chemotherapy and immunotherapy has been evaluated. Some evidence regarding the alleviating role of probiotics in chemotherapy side effects underscores the potentiality of probiotics for this purpose, but more clinical studies are warranted to increase the insight in this era. Also, some bacterial strains were found to have a positive role in response to immunotherapy in melanoma patients. However, there is no study in BC patients. In concomitant administration of probiotics with other therapeutic modalities, the metabolic activity of probiotics in metabolizing chemotherapeutics should be considered to prevent over/or under-treatment of anticancer agents (Silva et al. 2014).

Evidence regarding the preventive role of probiotics in BC is limited to three case–control studies which have shown that consumption of fermented milk products containing LAB and LcS were inversely associated with the risk of BC. However, no other probiotic strains were evaluated in terms of their preventive characteristics in BC. Owing to the mentioned facts, the efficacy of probiotics in BC treatment and prevention have not been confirmed yet. Also, beneficial strains in the prevention and treatment of BC have not been determined precisely. Apart from the mentioned facts, there is great concern regarding the safety of probiotics and also their efficiency in alteration of gut microbiota based on the reported individual differences in probiotics colonization ability. Table 8.2 summarizes the efficacy vs concern of probiotic consumption in prophylaxis and management of BC.

To sum up, the evidences are not solid for the preventive and curative role of probiotics in BC. However, due to the high relevance of gut microbiota and BC,

more clinical studies are needed to meticulously determine the beneficial strains, their safety, and underlying mechanism for this purpose. In the end, it seems that prescription of any regimen based on probiotics for BC prevention and/or treatment in the future should be personalized to meet the need arising from individual differences in response to probiotics.

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Probiotics for Management of Gastrointestinal Cancers

9

Nilesh Rai, Anurag Kumar Singh, Priyanka Kumari Keshri, Suvakanta Barik, Swapnil C. Kamble, Santosh Kumar Singh, Rajiv Kumar, Pradeep Mishra, Deepak Kotiya, and Vibhav Gautam

Abstract

Probiotics has been used for management of genetically predisposed health disorders associated with gastrointestinal (GI) tract. Administration of specific probiotics in defined doses causes revitalization of healthy gut microflora that can positively modulate immune response within GI tract and hence, aid in the management of inflammation of intestinal mucosa. Thus, it has potential of becoming a durable therapeutic approach to resolve metabolism related disorders including GI cancers. Probiotic-induced competition can exclude and replace pathogenic microorganisms from GI cancer-induced niche in gut. Notably, oral administration of probiotics is a key driving factor for the ease of management of post-operative complications of GI tract cancers. Here, we attempt to summarize the diversified knowledge of probiotics to utilize as therapeutic tool in prevention,

N. Rai · A. K. Singh · P. K. Keshri · S. K. Singh · R. Kumar · V. Gautam (✉)
Centre of Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India
e-mail: vibhav.gautam4@bhu.ac.in

S. Barik
Chemical Engineering Discipline, Indian Institute of Technology Gandhinagar, Gandhinagar, India

S. C. Kamble
Department of Technology, Savitribai Phule Pune University, Pune, India

P. Mishra
Division of Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden

D. Kotiya
Department of Pharmacology and Nutritional Sciences, University of Kentucky, Lexington, KY, USA

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progression and treatment of GI tract cancer, current challenges of probiotics in regulating GI cancer progression, and future perspectives.

Keywords

Probiotics · Gastrointestinal cancer · Carcinogen · Immunomodulation

9.1 Introduction

Gastrointestinal (GI) tract cancer is the fifth most often diagnosed cancer, and ranks third as a leading cause of cancer-associated deaths across the globe (Bray et al. 2018; Siegel et al. 2020). Despite the progress and advancements in cancer therapy, including radiotherapy, multi-drug therapy, and nanocarrier mediated targeting of cancer cells; there are lacunae in therapeutic approaches related to post-operative complications and toxicity.

Digestive tract of human contains trillions of bacteria. Approximately 400 different species have been identified so far (Lloyd-Price et al. 2016). These include beneficial as well as pathogenic species. The secretion of beneficial flora neutralizes the toxin produced by pathogenic bacteria, thereby protecting the normal functioning and maintenance of healthy gut. However, unpremeditated population imbalance towards dominance of pathogenic bacteria may lead to disease development such as gastric cancer (Ferreira et al. 2018). Genetic predisposition and unhealthy eating habits can further accentuate GI disorder. Genetic composition of host significantly contributes to the susceptibility of GI disorders and it is associated with neurological problems such as depression, autism spectrum disorders, and attention deficit hyperactivity (Ding et al. 2020; Nudel et al. 2020). Repeated occurrence of such incidences, if unchecked, may develop into disease. GI associated disorders include inflammatory bowel diseases (IBD), pathogenic infections of bacteria or virus, irritable bowel syndrome (IBS), and diarrhea associated with antibiotics (De Preter et al. 2011; Zuccotti et al. 2008). Alterations due to imbalance in normal gut flora can be counter checked through the intake of probiotics. Probiotics is a heterogeneous mixture of viable microorganisms that positively influence health outcomes by modulating the population dynamics of indigenous flora (Liang et al. 2019). Probiotics reduce symptoms associated with various GI disorders (Guarino et al. 2015; Mneimneh and Koleilat 2017), harmonize quantity and variety of advantageous gut microbiota (Irwin et al. 2018), revamp levels of cholesterol and lipid profile in blood (Guo et al. 2011; Sun and Buys 2015), reduce blood pressure and hypertension (Khalesi et al. 2014), remove mycotoxins (Nikbakht Nasrabadi et al. 2013), progress mental ability and cognitive function, and control diabetes through improving glucose tolerance of blood (Sun and Buys 2016).

In context of GI cancer treatment, post-operative complication of GI tract cancer can be managed by oral administration of functional food. The ease of administration of probiotics has attracted the scientific community in GI tract cancer to consider it as

a therapeutic tool. This can be corroborated with increase in publications on probiotics to treat GI tract cancer. Further, probiotics are being prescribed by gastroenterologists' to maintain health of GI tract (Draper et al. 2017). In this chapter, we have followed the utility of probiotic as potential therapeutic agent/adjuvant towards prevention, progression, and treatment of GI cancers.

9.2 Development of Gastrointestinal Cancer

Gastrointestinal cancer is a group of GI tract malignancies that includes cancers of organs of digestive system *viz*. esophagus, liver, gallbladder, pancreas, small intestine, colon as well as rectum (Pourhoseingholi et al. 2015). Table 9.1 describes the global burden of six most common GI cancers for both the genders (up to 2018). This corresponds to cumulative worldwide annual incidence and deaths by gastric cancer with nearly 10,00,000 new cases and approximately 8,50,000 deaths at the mean time, while esophageal cancer ranks seventh in terms of incidence that is 5,72,000 new cases and sixth in terms of mortality i.e. 5,09,000. Furthermore, collectively for both genders, colorectal cancer is also frequently diagnosed cancer with incidence is 10.2% of total cancer and 9.2% of total cancer deaths (Bray et al. 2018). The population based study of foremost mesenchymal tumors of GI tract i.e. GI stromal tumor represent approximately 5% of all sarcoma and <1% of all GI cancers, having speculated prevalence of 129 per million while the incidence was 14.5 per million in the USA (Corless 2014; Lim and Tan 2017). These data represent gastric cancer as the fourth most commonly occurred cancer and second most cause of cancer-related death after lung cancer (Bray et al. 2018; Ferlay et al. 2010). The third, fourth, and seventh frequently occurred cancers are colorectal, gastric, and esophageal cancers having approximately 1.4, 1, and 0.45 million first time diagnosed cases, respectively (Richman et al. 2017). Epidemiological data suggests

Table 9.1 Burden of new cases and deaths throughout the world due to GI tract cancer (up to 2018)

S. no.	Type of GI cancer	Number of new cases (% of total cancer)	Number of mortality cases (% of total cancer)	Rank (incidences of new cases)
1	Colorectal cancer	18,00,977 (10.2)	8,61,663 (9.2)	Third
2	Gastric cancer	10,33,701 (5.7)	7,82,685 (8.2)	Fourth
3	Liver cancer	8,41,080 (4.7)	7,81,631 (8.2)	Sixth
4	Esophageal cancer	5,72,034 (3.2)	5,08,585 (5.3)	Seventh
5	Pancreatic cancer	4,58,918 (2.5)	432,242 (4.5)	Twelfth
6	Gallbladder cancer	2,19,420 (1.2)	1,65,087 (1.7)	Twentieth

GI cancers to be chief global health problem and contributing to a cumulative incidence rate of about 25% of all cancer. It is responsible for 9% of total mortality by cancer worldwide (Ghoncheh and Salehiniya 2016).

The chief cause of GI cancer has been identified as chronic inflammation (Hanahan and Weinberg 2011). Inflammation promotes progression of gastric tumor and boosts up metastasis and invasion (Echizen et al. 2019). DNA damage in the epithelia of gut could be attributed through production of inflammatory cytokines (Hattori and Ushijima 2016). Cancer develops in three steps due to higher expression of interleukin-1,6,10,18 and tumor necrosis factor-α (TNF-α). These three steps are as follows:

- Stimulation of mitogen-activated protein kinases (MAPK) and Wnt signaling pathway, and activation of kappa light-chain enhancer of activated nuclear factor B (NF-κB) (Echizen et al. 2019);
- Apoptosis inhibition;
- Oxidative stress enhancement such as increased expression of reactive oxygen species (ROS) (Klampfer 2011).

The transformation of gut epithelial cells is significantly influenced by signal transducer and activator of transcription 3 (STAT3) upon sensitization through IL-6 and IL-11 (Putoczki et al. 2013). β-catenin forms a complex with adenomatous polyposis coli (APC), glycogen synthase kinase (GSK) 3β, and axin which leads to anomaly of Wnt pathway in epithelial cell and activation of proto-oncogenes cyclin D1 and c-Myc (Shitashige et al. 2008a, b). Therefore, factors associated with inflammation could activate oncogenes like Kirsten rat sarcoma viral oncogene homolog (KRAS) and tumor-suppressor genes like p53 could be inactivated (Raponi et al. 2008).

9.2.1 Role of Gut Flora in Development of Gastrointestinal Cancer

Healthy intestinal microflora is expressed through its dynamic, complex, and wide collection of microorganisms (Javanmard et al. 2018). GI microflora has been shown as most diversified, adaptable, and renewable metabolic representative of the body. Its activities and composition can impact both intestinal and systemic physiology. Several studies have speculated gut microbial dysbiosis to be major cause of carcinogenicity of GI cancer. GI cancer is multifactorial disease which includes gut microbiota, host, and various risk-driving factors that collectively lead to the process of carcinogenesis (Garrett 2015; Rawla and Barsouk 2019). Development of GI cancer has been associated with intestinal microbiota through accumulative production of toxic and genotoxic metabolites of bacteria, which has ability to direct the mutation in reactive oxygen species (ROS), hydrogen sulfide (H_2S), fructose-1,6-bisphosphate aldolase (FBA) (Bhatt et al. 2017; Nougayrede and Oswald 2011); by modulating the intracellular signaling pathways through peptides (act as quorum sensing molecules) produced by microbiota including *Escherichia coli, E. faecium,*

and *Bacillus* sp. (Wynendaele et al. 2015), toll-like receptors (TLRs)-mediated induction of pro-carcinogenic pathways (Fukata and Abreu 2008); T_H cell-mediated induction of cell proliferation (Marchesi et al. 2011); and binding specific cell surface receptors (Goodman and Gardner 2018).

Out of nearly 3.7×10^{30} microorganisms on earth, few have been identified through International Agency for Research on Cancer (IARC) as cancer causing agents. These cancer causing agents include *Streptococcus bovis, Helicobacter pylori*, *Clostridium, Bacteroides,* human papilloma virus (HPV), hepatitis B virus, hepatitis C virus, HIV type 1, human T-cell lymphotropic virus type 1, human herpes virus type 8, Epstein–Barr virus, and *Schistosoma haematobium* (Jahani-Sherafat et al. 2018; Plummer et al. 2016; Strofilas et al. 2012). Association of gut microbiota with three types of GI cancer—gastric cancer, esophageal cancer, and colorectal cancer—has been briefed upon (Table 9.2).

In contrast to cancer-inducing microbes, some specific strains of bacteria such as *Lactobacillus acidophilus* and *Bifidobacterium longum* inhibit tumorous growth of colon carcinoma (Chang et al. 2012; Drago 2019). Therefore, the homeostasis between "beneficial" and "detrimental" bacteria has inference in the settlement of cancer stage.

9.3 Probiotics: An Emerging Therapeutic Tool

Microbial infections are routinely treated by antibiotics. However, it wipes out major population of useful microbiota. In an alternative treatment method involving probiotics, such existing population can be sustained. The term "probiotics" emerged in 1974 as oral consumption of microorganisms for the promotion of health benefits generally after an adequate sum of supplementation. Beside whole organism, integral parts such as DNA or peptidoglycans of bacterial cell might have its own importance in probiotic effectiveness. The supplementation quantity to host has been suggested by the Food and Agriculture Organization (FAO)/World Health Organization (WHO) in 2002 (Hill et al. 2014). Probiotics impart a beneficial effect on gastrointestinal microbiota which leads to positive implication on host. This is achieved by improvement of barrier integrity, diminishing metabolism of pro-carcinogenic substances, and inhibiting growth of pathogens. It helps in building immunity, boost wound healing process, and diminishing inflammation (Oelschlaeger 2010). Probiotics have been reported to diminish oxidative stress molecules such as ROS, H_2S (Wang et al. 2017); and it enhances apoptosis through increased expression of butyrate (Moens et al. 2019; Pant et al. 2017).

Utilization of probiotic as therapeutic tool is a developing trend. It includes newer microorganisms and combinations, synbiotics (combined approach of probiotics and prebiotics), and is a personalized move towards outline of candidate microbes in diseases such as inflammation, obesity, and lipid metabolism. More specifically, administration of probiotics has effectively shown their efficiency and has provided strong therapeutic approach to treat problems like oral disease, immunological disorders, hair loss, IBD, cystic fibrosis (Mu et al. 2018), urogenital infections,

Table 9.2 Types of GI cancer and associated microbiota

S. no.	Type of GI cancer	Microbe/ s associated	Mechanism	References
1	Gastric cancer	*H. pylori*	Increased expression of IL-1, IL1β, IL-6, IL-7, IL-8, IL-10, and IL-18, TNF-α, IFN-γ Upregulated oncogenic signaling pathways (PI3K/Akt, ERK/MAPK, NF-κB, Wnt/β-catenin, and STAT3) Suppression of Tumor-suppressor pathways High p53 mutation	Bhardwaj et al. (2015), Doorakkers et al. (2016), Khatoon et al. (2016), Lv et al. (2019), Niu et al. (2020), Yong et al. (2015)
2	Esophageal cancer	Gram-positive bacteria (*Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Fusobacteria*, and *Proteobacteria*)	LPS mediated Upregulation of Immune responses, NF-κB Increased Inflammatory cytokines (TNF-α, IL1β, IL6, IL8) High Inducible NOS and NO	Lv et al. (2019), Xu et al. (2015), Yang et al. (2009)
		Enterobacteriaceae	Antibiotics and PPI alter the microbiome	Hu et al. (2017), Neto et al. (2016)
3	Colorectal cancer	Sulfate-reducing bacteria (*Desulfovibrio vulgaris*)	LPS and LDA mediated Transformation of primary bile into secondary bile acids	Canani et al. (2011)
		Fermentation of intestinal microbes (*Eubacterium rectale* and *Faecalibacterium prausnitzii*)	SCFAs mediated Suppressed Pro-inflammatory mediators (IL-1β, IL-6, iNOS, COX2, and TNF-α) Decrease in DNA methylation-mediated GPR109a silencing Downregulation of c-fos, p21 gene, and ERK1/ 2 phosphorylation	Bardhan et al. (2015), González-Sarrías et al. (2014), Koh et al. (2020)

eczema, and allergies (Abrahamsson et al. 2013). Considering the constant interplay of gut microbiota in physiology of a human, exploitation of probiotics in management of a number of GI related disorders, including IBD, IBS, antibiotic associated diarrhea and pathogenic viral or bacterial infection has been reported (De Preter et al.

2011; Zuccotti et al. 2008). Probiotics exert great impact on qualitative and/or quantitative modulations of GI microbiota.

Probiotics achieve these beneficial impacts through numerous mechanisms such as

- inhibitory substance production (bacteriocins and acid),
- anti-invasive effect,
- production of natural antibiotics,
- obstruction of pathogen adhesion,
- release of molecules with antioxidant property and,
- competition for nutrients.

In therapy of several disorders, bacteria having probiotic activity such as *Lactobacilli*, *Bifidobacteria*, non-pathogenic strains of *E. coli* and yeast like *Saccharomyces boulardii* have been reported (Klaenhammer 2000). Probiotics protect mucosal gut barrier integrity against the negative impact of *E. coli* in TLR-independent way through alteration of protein kinase C signaling (Zyrek et al. 2007).

9.3.1 Composition of "Probiotics"

Most of the microorganisms identified as probiotics for GI disorders therapy are gram-positive in nature (species of *Lactobacillus* and *Bifidobacterium*) (Marco et al. 2006). However, some of the gram-negative bacteria are also being utilized as probiotics. *Escherichia coli* Nissle 1917 (EcN) strain (Nissle 1959) is a gram-negative bacteria (also referred to as "Mutaflor") which has been exploited for treatment of colitis and chronic constipation in Germany (Möllenbrink and Bruckschen 1994). In dairy products, *Streptococcus thermophilus* and *Lactococcus lactis* are two most commercially used important lactic acid bacteria (Felis and Dellaglio 2007).

Probiotic application is usually strain-specific or species-specific (Bron et al. 2013), that is each strain produces beneficial effects against a specific disorder and may not be effective against another. Further, combined administration of probiotics and prebiotics (i.e. synbiotics and combination of more than two probiotics) could be highly efficient than single probiotics.

9.4 Management of Gastrointestinal Cancer Using Probiotics

Aptitude of probiotics to amend the host immune response and intestinal microbiota suggests its utility in treatment of cancer as an adjuvant. The therapeutic efficacy of probiotic in treating, preventing, and diminishing the succession of various kinds of cancers like colorectal, breast, liver, bladder, cervical, and colon in cancer patients has been shown.

9.4.1 Prevention

Cancer preventive function of probiotics has been demonstrated through epidemiological data (Kumar et al. 2010b). The gut microbiota of cancer patients can be reflourished by post-probiotics administration, which improves re-establishment of functionality and enrichment of commensal gut bacteria. Intestinal microbiota can be manipulated by oral consumption of probiotics to advance safety issue, and reduce severe side effects of GI tract (Mego et al. 2013). The *in vivo* and *in vitro* studies have remained strong evidence of utilization of probiotics in avoidance of GI tumor. Number of bacterial strains have been reported to show anticancer effect which belong to genera *Lactobacillus* (L) and *Bifidobacterium* (BF), and strains of *Enterococcus, S. thermophilus, Saccharomyces boulardii,* and *E. coli* (Fotiadis et al. 2008; Serban 2014). *In vitro* studies have shown that this microbiota leads to mortality of different cancer cells including MCF-7, HeLa, AGS, Caco-2, and HT-29 (Nami et al. 2015). Production of short chain fatty acids (SCFAs) has been reported to be associated with the anti-inflammatory and suppressive effect of probiotics, which could be supported by enhancement of pathway related to SCFAs (Lee et al. 2016; Morrison and Preston 2016).

9.4.2 Progression

One of the major risk factor of cancer is chronic inflammation (Mantovani et al. 2008). IBD is a risk factor for colon cancer and inflammatory conditions, like those induced by hepatitis B, and hepatitis C virus infection could lead to risk of hepatocellular carcinoma (HCC) (Ashtari et al. 2015). Inflammation not only induces risk of colon cancer associated with colitis, it may also lead to progression to sporadic colon cancer (Grivennikov et al. 2012; West et al. 2015). Inflammation induces aberrant tissue repair, genotoxicity, invasion, metastasis, and proliferative responses. The foremost inflammatory pathways concerned in carcinogenesis converge at NF-κB and STAT3 (El-Deeb et al. 2018).

9.4.3 Treatment

Studies performed in colon cancer (Drago 2019; Weidong et al. 2019) and gastric cancer (Liong 2008; Rafter 2004) has speculated that probiotics acquire pro-apoptotic and anti-proliferative effect in GI cancer. Study on colon cancer cell lines—SW 480, HT-29, and Caco-2—and gastric cancer cell reveals that probiotics *Bifidobacterium adolescentis* SPM0212 and *Lactobacillus rhamnosus* strain GG (LGG) have anti-proliferative impact (Arian et al. 2019; Bahmani et al. 2019). *Enterococcus lactis* IW5 obtained from human gut has been reported to inhibit pathogenic bacterial growth. Studies on lactic acid bacteria (LAB) have suggested that bacteria produce bacteriocins or a soluble compound that may directly interact with cancer cell and inhibit their growth in culture. These effects have also been

reported in colon cancer, stomach cancer, and non-GI tract cancers such as cervix, breast, and myeloid leukemia cells (Ghoneum and Gimzewski 2014; Kim et al. 2008; Rossi et al. 2018).

Probiotics act by controlling the assembly of inflammatory molecules (such as interferons, interleukins, and cytokines) (Le Leu et al. 2005). Probiotic supplementation of LGG has been reported to avert carcinogenesis of colon by suppression of NF-kB pathway (Jacouton et al. 2017) (a pro-inflammatory pathway associated with IBD and colon cancer). Exopolysaccharide LA-EPS-20079 purified from *L. acidophilus* inhibits colon cancer through molecular regulation of both NF-κB inflammatory pathways and apoptosis (El-Deeb et al. 2018; Zhuo et al. 2019). The *in vitro* studies on probiotic *L. reuteri* show that it works in dose-dependent manner and inhibits cell proliferation significantly. It suppresses gastric cancer cell invasion through downregulating pathways of extracellular matrix degradation like urokinase-type plasminogen activator (uPA) and urokinase-type plasminogen activator receptor (uPAR) (Rasouli et al. 2017) and cell survival (Bcl-2, Bcl-xL) and proliferation gene (Cox-2, cyclin D1) that are regulated by downregulation of NF-κB-dependent genes (Lee et al. 2008) (Fig. 9.1).

Multiple animal and human models have been proposed that suggest various mechanisms of GI cancer therapeutics (Fig. 9.2) through probiotic utilization. It includes the following:

- Gut microbiota modulation,
- Enrichment of functions of gut barrier,
- Protection of DNA damage after deterioration of potential carcinogens in intestinal epithelium, and
- Upregulation of immunity and inflammatory system of individual.

9.4.3.1 Modulation of Gut Microbiota

Probiotics can modulate gut microbial harmony. It could lead to maintenance of equilibrium and diminishing of any enlargement of detrimental or cancer-inducing gut microbiota. For example, the growth of gram-negative bacteria is inhibited under reduced intestinal pH that is contributed by gram-positive probiotic synthesized antimicrobial peptides, lactic acid, and propionic acid (Šušković et al. 2010). Similarly, strains of *Lactobacilli* have functional rivalry against gram-negative *H. pylori* which is associated with gastric cancer (Chen et al. 2012). Li et al. suggested that probiotics shift the composition of gut microbiota towards monotonous condition for beneficial bacteria, e.g. *Oscillibacter* and *Prevotella* (Li et al. 2016). Both of these produce anti-inflammatory metabolites that decrease T helper 17 (Th17) polarization, and favor anti-inflammatory T_{reg}/Type 1 regulatory T (Tr1) cell differentiation in the gut (Nazir et al. 2018).

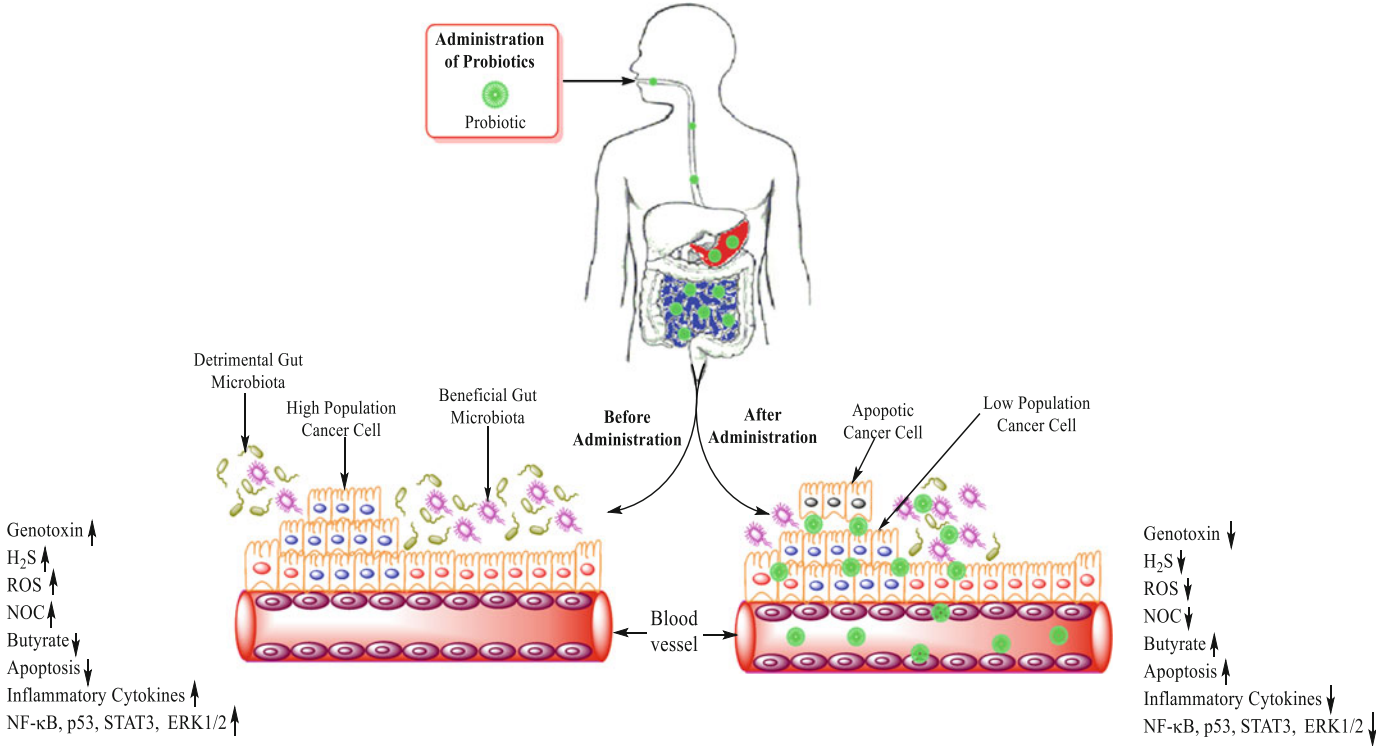

Fig. 9.1 Route of probiotic administration and its effect on gastrointestinal cancer. Toxins produced by detrimental bacteria cause gut microbiome imbalance, which leads to altered expression of various genes towards GI cancer. Oral administration of probiotics leads to maintenance of gut microbiota. Further, these microbiota and probiotics enhance apoptosis and butyrate production and decrease the inflammatory cytokines such as IFN-γ, TNF-α, and interleukins; and in addition, decreases the levels of STAT3, VEGF, ERK1/2, NF-κB, p53, genotoxin, and maintain the MAPK/Akt, Wnt signaling pathways

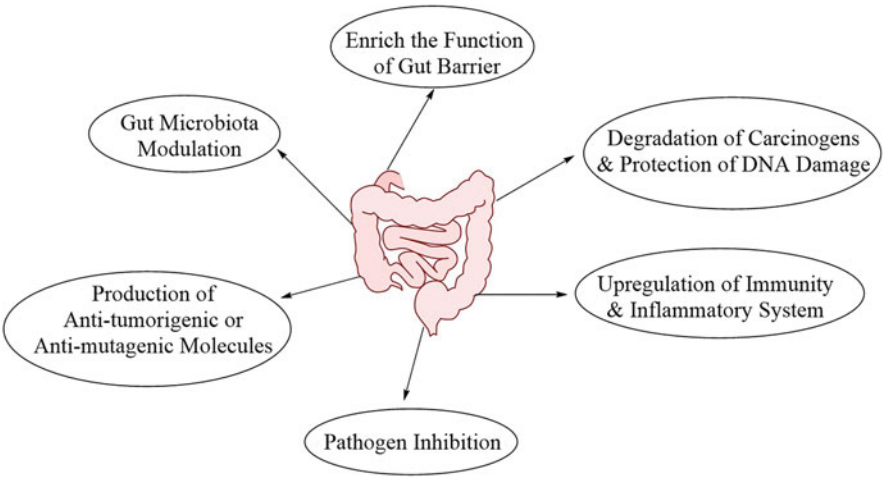

Fig. 9.2 Potential mode of action of probiotics for the prevention and therapeutics of gastrointestinal cancer. The possible anticancer mechanism of probiotics involves (1) gut microbiota modulation, (2) enrichment of functions of gut barrier, (3) protection of DNA damage after deterioration of potential carcinogens in intestinal epithelium, (4) upregulation of immunity and inflammatory system of individual (5) pathogen inhibition and (6) production of anti-tumorigenic and anti-mutagenic molecules

9.4.3.2 Enrichment of Functions of Gut Barrier

Gut microbiota dysbiosis is associated with interruption of physiological communication between microbiota and epithelial cells. This results in violating epithelial barriers, encouraging inflammatory pathologies that could cause cancer initiation and progression (Roy and Trinchieri 2017). Fermented products of probiotics prevent disturbance of epithelial barrier of intestine (Commane et al. 2005). Probiotic administration enhances function of intestinal gut barrier through increasing the tight junctions' protein expression *viz*. mucin gene 2 and 3 (MUC2 and MUC3) (Bermudez-Brito et al. 2012). *Lactobacillus plantarum*, as probiotic, reduces the downregulation in trans-epithelial resistance of Caco-2 cells (Ko et al. 2007).

9.4.3.3 Protection of DNA Damage After Deterioration of Potential Carcinogens in Intestinal Epithelium

Carcinogens like N-nitrosodimethylamine (NDMA) and 2-dimethylhydrazine (DMH) alter DNA sequence. Rat intestinal tumor induced by DMH showed limited growth in colon (as compared to control group of rat) after probiotic supplementation containing LGG, *L. acidophilus*, *S. thermophiles* DD145, and *Bifidobacterium animalis* CSCC1941 strains (Lee et al. 2020; McIntosh et al. 1999). Probiotics diminish DNA adduct formation or DNA damage induced by mutagen (Kumar et al. 2010a). An *in vitro* report on intestinal epithelial cells of rat demonstrated defensive action of probiotics against loss of intestinal barrier function and apoptosis of enterocytes because of 5-fluorouracil (5-FU) (Prisciandaro et al. 2012).

9.4.3.4 Upregulation of Immunity and Inflammatory System of Individual

Supplementation of probiotics having *Lactobacillus casei* strain Shirota increases natural killer (NK) and T cell activities. It advances macrophages phagocytic activity that inhibits cancer progression (Foo et al. 2011; Yamazaki et al. 2000). Translational research on colon cancer patients showed that oral administration of *Bacillus polyfermenticus* induces immunoglobulin G (IgG) formation and also alters cell number of $CD4^+$, $CD8^+$, or NK cells (Rossi et al. 2018).

9.5 Route for Probiotic Administration

Physiological character of probiotic has a deterministic effect on human health. Therefore, we need to elucidate most preferred route of administration for their excellent feasibility and viability for probiotic effectiveness.

9.5.1 Oral Administration

Oral consumption of probiotic plays a crucial role in strengthening mucosal immune barrier of gut through modulation of gut microbiota. Oral administration of probiotics influences the antimicrobial activity in intestine, by killing gram-negative and gram-positive pathogens thereby strengthening integrity of intestinal barrier (Cazorla et al. 2018). Oral administration of combinatorial strain of probiotics including *L. casei, S. faecalis, B. brevis,* and *L. plantarum* produce several beneficial traits (Chapman et al. 2012). Tumor-associated antigens (TAAs) could easily be delivered in form of orally consumed vaccine along with the probiotics. For example, administration of *Bifidobacterium* expressing Wilms' tumor 1 (WT1) protein (Kitagawa et al. 2017). Oral administration of *B. longum* strain, used as a delivery system for endostatin gene (*B. longum*-En), in tumor-bearing nude mice results in strong inhibition of growth of solid liver tumor (Fu et al. 2005).

Clinical trial on patients of colon cancer for probiotic strains *B. longum*-88, *L. acidophilus*-11, and *L. plantarum* CGMCC administered orally for pre- and post-operative at different dose and duration was conducted. The results showed that probiotic administered cohort had inhibition of p38 mitogen-activated protein kinase (MAPK) signaling pathway and decreased duration of post-operative pyrexia, rate of post-operative infectious complications, serum zonulin concentration, and duration of antibiotic therapy (Liu et al. 2012).

9.5.2 Nasal Administration

Vaccination with probiotics strain *Lactococcus lactis* to C57BL/6 mice model of E7-expressing tumor growth through intranasal, showed anti-tumor effect by increasing interferon-γ (IFN-γ), E7-specific T-cell proliferation, and cytotoxic

activity (Gomez-Gutierrez et al. 2007). Effect of probiotic bacteria *Lactobacilli*, has been assessed in providing protection against common cold or pneumonia. Patients of bronchoalveolar lavages (BAL) receiving probiotics by nasal along with oral route had maximum levels of immunoglobulin G and immunoglobulin A anti-PppA antibodies (Vintini et al. 2010). However, the intranasal administration of probiotics in case of GI cancer has not been well elucidated so far.

9.5.3 Subcutaneous Administration

B. Sheil and his group challenged the conservative theory of probiotics after subcutaneous administration of *L. salivarius* UCC118, instead of mucosal administration into knockout mice for interleukin-10 (IL-10) (Sheil et al. 2004). They did not find any specific anti-inflammatory effect, thereby suggesting that probiotics has local anti-inflammatory effect.

9.6 Challenges of Probiotics in GI Cancer Treatment

1. Probiotics act at multiple levels—individual *versus* colonial microbes, neutralization of toxins by macromolecules released *versus* those ingested, single strain *versus* multiple strains. Thus, obtaining correct configuration of this plethora of probiotic strain needs to be deciphered in a more elaborate way.
2. The complexity of multiple pathways cross-linked to gut epithelia and microbiota in a niche, is enormous. Hence, identification of key signatures that may be considered as potential predictive marker in GI cancer treatment is a perplexing task.
3. Commercially available probiotic supplements have a fixed composition that may not be suitable for different stages, grades, age, and gender of GI cancer patients worldwide.
4. GI cancer is multifactorial disease. More clinical trials involving multiple control groups of combinatorial therapy involving different microbiota along with immunotherapy or chemotherapy are required.

9.7 Conclusion and Future Perspective

GI cancer progression is influenced by various factors. It develops as a result of intricate association between epigenetics and genetics, immunological, diet, lifestyle, and environmental factors through which gut microbiota interacts and leads to the tumorigenesis and growth. Current research throws notable feature of probiotic (especially from the genera *Lactobacillus* and *Bifidobacterium*) and synbiotics (combination of probiotic and prebiotic) exertion of significant anti-carcinogenic effect. However, there are limited studies and reports that have strong correlation of biotherapeutics in prevention or therapy of GI cancers. Nonetheless, the results have

captured attention towards genetic maneuvering of probiotics, deliberated to play a role of delivery system for pro-apoptotic or anti-proliferative factors (superoxide dismutase, SCFA, catalase, TGF-β, IL-10) in GI tract. In addition, simplicity of oral intake of probiotic should be kept in mind during selection of preferred administration routes.

Furthermore, the composition of human gut microbiome is temporally dynamic that necessitates evaluation through clinical trials. Data regarding efficacy of probiotics, safety, duration of probiotic therapy, and the optimal doses are still in deficit. Administration of mixture of probiotics, synbiotics with their pros and cons along with recurrence post-probiotic treatment needs to be elucidated in future. The interactions of gut microflora and probiotics with immunologic and genetic factors, diet, and age need to be deciphered before drawing any definite conclusion. These are few of the many unanswered queries for which responses are expected in coming years. Therefore, researchers must continue to explore towards accomplishing these queries before GI cancer treatment through probiotics becomes a norm.

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Potential of Probiotics in the Management of Lung Cancer

10

G. Divyashri, T. P. Krishna Murthy, and Manikanta Murahari

Abstract

Around the world, growth of lung cancer remains to be the most widely recognized reason for disease-related deaths. It is the second most common cancer observed in both men and women with a risk of 1 in 15 for men and 1 in 17 for women as per the statistical data of American Cancer Society. Probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer health benefits on the host." An abundance of information has demonstrated that probiotics residing in the gut play a vital role in tumor prevention and treatment of different types. Metabolites produced by these beneficial microorganisms regulate the gut immunity and impact distal organs like lungs. Thus, bi-directional communication between gut and lung (termed as Gut–Lung axis) is better characterized by intestinal disturbances seen in lung diseases. Emerging studies indicate that probiotics supplementation exert a wide range of beneficial effects on humans in the attenuation of perception of cancer symptoms and disease duration. Furthermore, clinical trials of several probiotics have claimed therapeutic benefit, but the exact mechanism is not clearly understood. The present chapter summarizes the experimental or clinical studies conducted with a note on application of probiotics along with recent findings.

G. Divyashri · T. P. Krishna Murthy
Department of Biotechnology, M S Ramaiah Institute of Technology, Bangalore, Karnataka, India
e-mail: krishnamurthytp@msrit.edu

M. Murahari (✉)
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, M.S. Ramaiah University of Applied Sciences, Bangalore, Karnataka, India
e-mail: manikanta.py.ph@msruas.ac.in

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Lung cancer · Probiotics · Gut–Lung axis · Bi-directional communication · Clinical studies

10.1 Introduction

For quite a few decades, lung cancer has been the most commonly occurring cancer worldwide, contributing to the largest percentage of all cancer-related deaths (Jones and Baldwin 2018). A few epidemiological perceptions performed crosswise over differed statistic accomplices in India affirm the noteworthy rise of lung cancer, significantly contributing towards the cancer morbidity and mortality (Noronha et al. 2016). In India estimated lung cancer mortality in 2018 was 67,795 with 48,698 new cases reporting in men and 19,097 new cases in Indian women (Bray et al. 2018). Smoking tobacco is found to be the chief hazard factor for causing lung cancer in Indian men; however, among Indian women, the relationship with smoking is not solid, recommending that there could be other hazard factors other than smoking (Noronha et al. 2016). Besides, we have a limited understanding of the influence of the various factors viz., presence of indoor air pollution, the use of domestic or biomass fuel exposure, the presence or lack of micronutrients in our diet, occupational exposure, and the possible impact of infectious pathogens such as *Mycobacterium tuberculosis* on the occurrence of lung cancer. Furthermore, there is a lack in our present comprehension of the varying epidemiological patterns of lung disease among Indian patients. While the worldwide pattern of an ascent in adenocarcinoma seems, by all accounts, to be paralleled in India, we do not understand the alarming rise in the incidence of lung cancer (Noronha et al. 2016). In spite of many advancements are made in recent years towards the development of diagnostic methods, the outcomes of lung cancer patients remain poor due to molecular changes and therapeutic interventions. Therefore, a better understanding of the risk factors may impact the preventative measures to be implemented at a community level. However, despite many advances in therapeutics, it has been reported that the overall 5-year survival rate is confined to 15% for men and 21% for women (Street 2018). Therefore, innovative approaches are necessary in prevention and treatment of lung cancer. In line with this, these days it has fascinated the scientific community about the protective nature of probiotics against various cancers. Several scientific reports are available related to epidemiological evidence in the use of probiotics in the prevention and treatment of various types of cancers (Nazir et al. 2018). The possible pleiotropic health effects of probiotics in eliciting anti-microbial and anti-tumor effects is by delaying tumor growth by enhancing host immunity (innate and adaptive) via degradation of mutagens, competitive inhibition of foodborne pathogens, and heavy metal sequestration (Javanmard et al. 2018). However, many other beneficial modes of action of probiotics remain unknown. In addition, limited number of scientific reports are available to explain the link between probiotics and lung cancer. Considering the importance of probiotics, this chapter

was an attempt to discuss the recent developments and understandings on the direct effects of probiotics in lung cancer treatment. It is also intended to provide insights on the indirect possible roles and impact of probiotics on various respiratory diseases and their probable mechanisms of action in the lung cancerous cell.

10.2 Probiotics and Gut–Lung Axis

Mammalian gastrointestinal tract (GIT) is a metabolically active organ comprising a diversity of microbial species. This commensal intestinal microbial species is essential as they protect the host against infections and maintain the body's homeostasis under normal circumstances (Divyashri et al. 2015). Among the various intestinal microorganisms, *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* have proven to influence the host beneficially by improving the intestinal microbial balance and thus are classified as "Probiotics." The usage of term "Probiotics" was first introduced by Parker in 1974, defining them as "organisms and substances that pose a beneficial impact on the host animal by contributing to its intestinal microbial balance" (Fuller 1999). Since then, several times the definition for probiotics has been improved. Food and Agriculture Organization of the United Nations World Health Organization defines them as beneficial microorganisms which when administered in sufficient amounts confers health advantages to host organisms (FAO/WHO 2001). The conventional source of probiotics recommended by FAO/WHO for human use is the GIT. Quantity of microorganisms inhabiting the GIT has been evaluated to surpass 10^{14} and a larger portion of them belongs to the domain Bacteria. Strikingly, every organism presents a particular "Microbial fingerprint," which is assorted by a variety of factors such as maternal environment, host genotype, diet, and antibiotic treatment (Hugerth and Andersson 2017). Even though the microbial composition varies from one individual to other individuals, compiled information from the studies pertaining to Human Microbiome Project, recognized species (2172) isolated from humans that are classified into different divisions. Divisions found include Proteobacteria, Fusobacteria, and Verrucomicrobia. Species belonging to Archaea (mostly *Methanobrevibacter smithii*), Eukaryotes (protists and yeasts), and Viruses are found to be less predominant. Bacterial taxa (~90%) found in human GIT are categorized into three divisions viz., Bacteroidetes (Porphyromonas, Prevotella), Actinobacteria (Bifidobacterium), and Firmicutes (Ruminococcus, Clostridium, and Eubacteria). Bacteria belonging to class Bacilli, including *Lactobacillus* spp., *Enterococcus* spp., and *E. coli* constitute the rest of the Firmicutes phylum (Behnsen et al. 2013). Bacteria in GIT (Gut bacteria) play a vital role in human health including improvement in digestion, vitamin B synthesis, and promotion of angiogenesis and nerve function (Zhang et al. 2015). Furthermore, alteration to this gut microbiota can be deterimental when the gut biological system experiences extreme unusual changes. In any case, changes in the gut microbiota can prompt numerous diseases in animals and humans (Nakamoto et al. 2017). Modification of gut microbiota using probiotics has gained importance due to its potential in treatment for several diseases in animals and humans. Although probiotics have long been

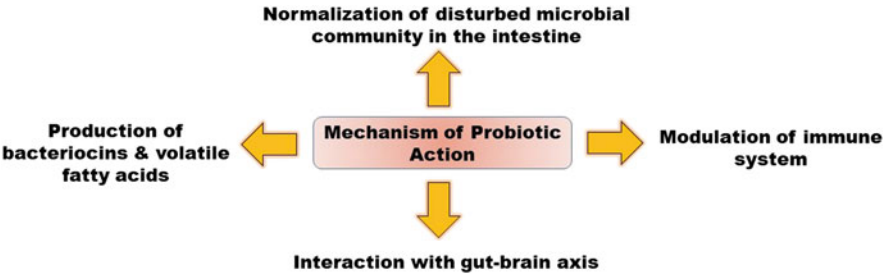

Fig. 10.1 Mechanism of probiotic action (Image courtesy: Plaza-Diaz et al. 2019)

used for human health improvement, even today, researchers continue to isolate and develop new probiotic species irrespective of their isolation from conventional sources (Azad et al. 2018). The capability of probiotics to impart health benefits has provoked increased scientific interest for a very long while. They are known to reduce and improve digestive disorders viz., acute, nosocomial, and antibiotic-associated diarrhea; *Clostridium difficile*-associated diarrhea and some inflammatory bowel disorders in adults; and allergic disorders such as atopic dermatitis (eczema) and allergic rhinitis in infants (Khalesi et al. 2019). Furthermore, probiotics are of interest as coadjuvants in the treatment of metabolic disorders, including obesity, metabolic syndrome, non-alcoholic fatty liver disease, and type 2 diabetes. Scientific evidence from animal and human investigation has illustrated the potentially favorable benefits of these microorganisms (Khalesi et al. 2019). However, the mechanisms of action of probiotics have received little consideration. Accumulating evidence demonstrates that these organisms offer health benefits by normalizing the disturbed microbial communities in the intestine by colonization, production of anti-microorganism substances such as bacteriocins and volatile fatty acids for competitive exclusion of pathogenic microorganisms, modulation of the immune system, and interaction with a gut–brain axis for maintaining normal homeostasis (Plaza-Diaz et al. 2019) (Fig. 10.1).

Lactobacillus acidophilus, L. rhamnosus, *L. reuteri*, *L. casei*, and *Bifidobacteria* spp. are commonly employed probiotic microorganisms. Strains of *Escherichia coli*, *Bacillus coagulans*, and certain *Enterococcus* spp., particularly *Enterococcus faecium* (SF68), and the yeast *Saccharomyces boulardii* are also used. The most significant findings of various research studies on the effect of probiotic species and strains on the modulation of the gut microorganisms in various models and diseases are presented in Table 10.1.

Symbiotic equilibrium between host and gut microbiota is highly sensitive to various intrinsic and environmental factors, including the use of antibiotics, host genetic background, diet quality, and the presence of allergens or infectious agents. All these factors can interrupt the composition of gut microbiota and lead to a state of "dysbiosis" (Levy et al. 2017). This dysbiosis in gut microbiota is associated with respiratory infections and lung disorders (Shukla et al. 2017). Also, alteration in lung

Table 10.1 Influence of probiotic species and strains on the modulation of the gut microbiota in various diseases models

Probiotic bacterial strain	Disease model	Disease	Research outcomes	References
L. acidophilus ATCC 4356 and *L. delbrueckii* subsp. *bulgaricus* DSM 20080	CD-1 Swiss albino mice	Oxidative stress and liver fibrosis	↓ Matrix metalloproteinases 9 in liver tissue ↓ Oxidative stress markers	El-Khadragy et al. (2019)
L. acidophilus	Eight-week-old male C57BL/mice	Inflammatory bowel disease	↑ IL-10, Treg ↓ IL-6, IL-1β, IL-17	Park et al. (2018)
L. acidophilus	Female BALB/c mice	Crohn's disease	↑ IL-17 ↓ Th17 function, IL-23	Chen et al. (2015)
L. acidophilus	BALB/c mice	Ulcerative colitis	↑ Lactobacilli, Bifidobacterium ↓ S. aureus	Chen et al. (2013)
L. casei BL23	Female C57BL/6 mice	Colorectal cancer	↑ Th17, Th 22, IL-10, and IL-22 ↓ Treg	Lenoir et al. (2016)
L. acidophilus NCK2025	Generation of TS4Cre × APClox468 mice	Colorectal cancer	↑ IL-10, IL-12 ↓ Treg	Khazaie et al. (2012)
L. fermentum FTDC 812	Eight-week-old BALB/c mice	Hypercholesterolemia	↑ Lactobacillus	Lye et al. (2017)
L. johnsonii	Male C57BL/6 mice	Acute live injury	↑ IL-22, Lactobacillus	Nakamoto et al. (2017)
L. plantarum CCFM10, RS15-3	58-week BALB/c mice	Oxidative stress	↑ Bacteroidetes, Firmicutes	Zhao et al. (2018)
L. acidophilus, B. cereus, B. infantis	Eight-week SPE male	Non-alcoholic fatty liver disease	↑ E. coli, Enterococcus ↓ Bifidobacterium, Bacteroides, and Lactobacillus	Xue et al. (2017)
L. acidophilus, L. rhamnosus, B. bifidum	Eight-week C57BL/6 mice	Type 2 diabetes	↑ Firmicutes, Actinobacteria ↓ Bacteroidetes	Bagarolli et al. (2017)

(continued)

Table 10.1 (continued)

Probiotic bacterial strain	Disease model	Disease	Research outcomes	References
L. brevis KLDS 1.0727 and *L. brevis* KLDS 1.0373	C57BL/6 mice	Type 1 diabetes	↓ Blood glucose levels	Abdelazez et al. (2018)
Bifidobacteriaceae, Lactobacillaceae, and Streptococcus thermophilus	NOD mice	Type 1 diabetes	↑ Gut microbiota composition ↓ IL-1β expression	Dolpady et al. (2016)
B. breve IPLA20004	Human colon	Inflammatory	↑ IL-8, IL-10, IL-12	Sanchez et al. (2015)
E. coli Nissle 1917	Male C57BL/6J mice	Chronic inflammation	↑ IL-10, tight-junction ↓ IL-17	Secher et al. (2017)
S. boulardii	Adult BALB/c mice	Acute liver failure	↑ Bacteroidetes ↓ Firmicutes, Proteobacteria	Yu et al. (2017)
S. boulardii	Six-week C57BL/6 mice	Type 2 diabetes	↑ Firmicutes, Proteobacteria, and Fibrobacteria	Everard et al. (2014)
E. hirae	C57BL/6J mice	Cancer	↑ Th 17 cell response	Daillere et al. (2016)
E. faecium CFR 3003	CFT-Swiss male mice	Oxidative stress	↓ Oxidative stress markers ↑ Activities of antioxidant enzymes ↑ Gamma-aminobutyric acid	Divyashri et al. (2015)

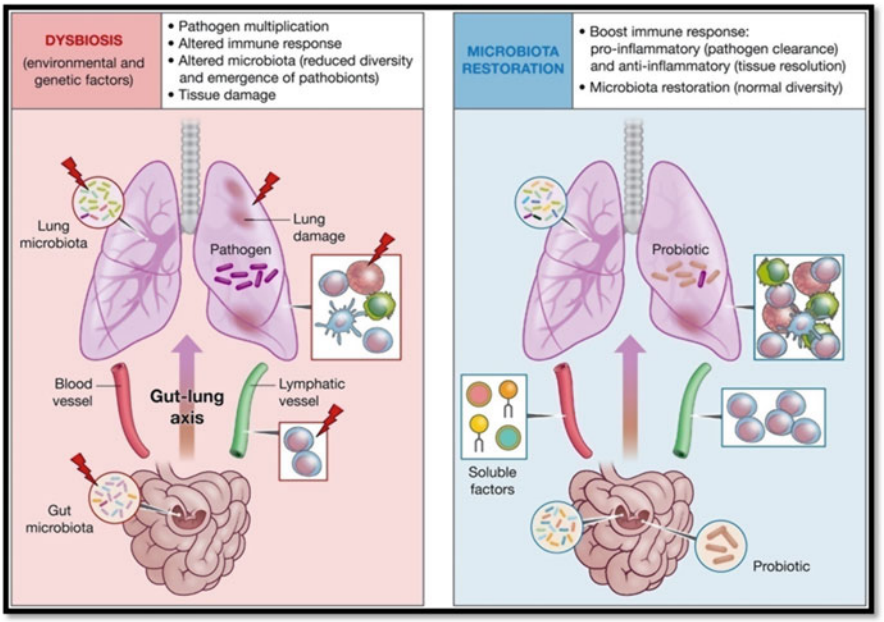

Fig. 10.2 Model of the host–microbiota interaction during dysbiosis through "gut–lung axis." Gut–lung axis refers to the cross-talk between these two mucosal sites of the body (Dumas et al. 2018)

microbial community (low density, at 10^3–10^5 CFU/g of lung tissue) is known to influence the composition of gut microbiota and dysbiosis in lung microbiota accomplishing the disturbances in gut microbiota. Emerging experimental evidence highlights a crucial cross-talk between gut microbiota and the lungs, termed the "gut–lung axis" (Fig. 10.2) (Dang and Marsland 2019). Mouse model studies demonstrated that the amputation of sensitive gut bacteria after administration of neomycin leads to an increase in the susceptibility to influenza virus infection in the lungs (Looft and Allen 2012; Ichinohe et al. 2011). Infection of the respiratory tract with influenza virus in mice model increased Enterobacteriaceae and reduced the levels of Lactococci and Lactobacilli in the gut microbiota (Looft and Allen 2012). Furthermore, upon administration of Lipopolysaccharide (LPS) in mice the dysbiosis in lung microbiota was accompanied by disturbances in their gut microbiota because of the movement of bacteria from their lungs into the blood-stream (Sze et al. 2014). All the aforementioned scientific evidence substantiates that gut and lung are intricately associated organs that control each other's homeostasis.

10.3 Potential of Probiotics in the Treatment of Lung Cancer

Scientific evidence claims that the genetic factors, alcohol intake (Druesne-Pecollo et al. 2014) smoking and pollution from transport (Vineis et al. 2006), and exposure to asbestos and silica dust (Islami et al. 2015) majorly accounts for the incidence of lung cancer. However, smoking is the major cause and accounts for 80–90% of all lung cancer cases (Alberg et al. 2013). In recent decades, significant improvements in the health of patients with early-stage of lung cancer are reported. However, in addition to many recent therapeutic interventions, the overall 5-year survival rate is confined to 15% for men and 21% for women (Sharma et al. 2018). This necessitates the need to look for alternative and innovative strategies to prevent and treat lung cancer.

Probiotic bacteria have recently become the focal point of research on account of their anti-cancer properties, therefore, their protective role against various cancers has fascinated the scientific community (Motevaseli et al. 2017). Fundamental mechanisms for their anti-cancer property are versatile including suppression of bacterial growth that is implicated in the production of mutagens and carcinogens, alteration in carcinogen metabolism, protection of DNA from oxidative damage, and regulation of immune system (Abedin-Do et al. 2015). Besides, they also possess the ability to change the expression of different genes that participate in cell death and apoptosis (Motevaseli et al. 2013), invasion and metastasis (Nouri et al. 2016), cancer stem cell maintenance, and cell cycle control (Modarressi et al. 2014). Furthermore, studies have demonstrated their modulatory effects on the cancer-related signaling pathways in a cell-type-specific manner (Taherian-Esfahani et al. 2016). These days, considerable attention is given to the utilization of probiotics in the treatment of lung cancer. However, only a few scientific literatures are available about the link between probiotics and lung cancer (Sharma et al. 2018). Probiotics are known to prevent and or treat lung cancer either by their direct mode of action or by their indirect possible roles (Fig. 10.3).

10.3.1 Direct Ways of Probiotic Action in the Treatment of Lung Cancer

The efficiency of a probiotic strain, *Lactococcus lactis* KC24 was evaluated for its anti-cancer effect on various cancer cell lines, including lung carcinoma (SK-MES-1) cell lines. Results showed 10^6 CFU of *L. lactis* KC24/well on various cell lines demonstrated strong inhibition of proliferation using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and proliferation of SK-MES-1 was inhibited by 86.53% (Lee et al. 2015). In similar lines, treatment of SK-MES-1 cell lines with 10^6 CFU/well of *L. lactis* NK34 resulted in strong inhibition of proliferation (Han et al. 2015). It can be noted that the above said two strains of *L. lactis* viz., KC24 and NK34 demonstrated a strong cytotoxic effect on lung carcinoma cell line (SK-MES-1), thereby encouraging the

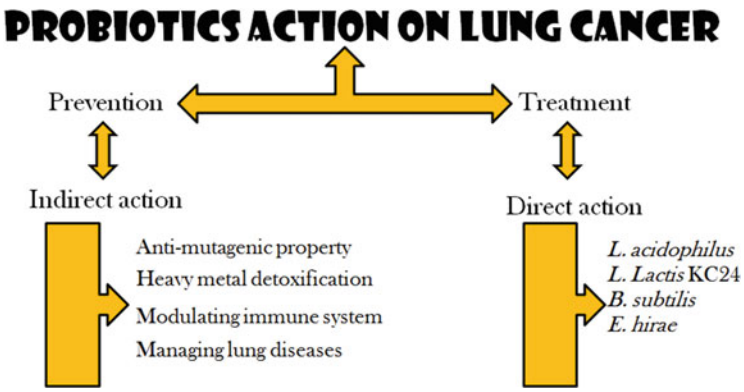

Fig. 10.3 Mode of action of probiotics on lung cancer either by their direct way or by their indirect possible roles

potential use of these strains in the microbial therapy for lung cancer treatment (Sharma et al. 2018).

Mice (C57BL/6J) with Lewis lung cancer (LLC) was utilized as a tumor model to evaluate the positive role of *L. acidophilus* to represent the use of probiotics (Gui et al. 2015). The study exhibited an increase in anti-cancer activity in *Lactobacillus*-co-treated mice (co-treated with an anti-cancer drug, cisplatin). It was observed that interferon (IFN)-γ, Gzmb, and Prf1 mRNA expression was upregulated. Earlier to this, the efficacy of anti-cancer vaccine either with probiotic strains viz., *E. faecium* K-50 and *S. cerevisiae* 14 K or with their metabolic yields was assessed for solid sarcoma 37 (S37) and metastatic Lewis lung carcinoma (3LL). Reports showed that combined administration of anti-cancer vaccine and probiotics could enhance and synergize the therapeutic benefit in both S37 and 3LL animal models. Furthermore, this collective combination could inhibit the metastasis by 2 to 2.5 times compared to the animals treated by vaccine alone under control group (Tanasienko et al. 2005). In line with this, a recent study of co-administration of milk which is fermented by *L. casei* CRL 431 to mice model with cancer BALB/c brought reduced tumor growth and lung metastasis. The observed beneficial effect might be because of activation of immune response like decreased infiltration of macrophages and increased $CD4^+$ and $CD8^+$ immune cells (Aragon et al. 2015). Another scientific learning connected to lung metastasis recognized that the use of probiotic including fermented milk product, Kefir in a BALB/c mice results exhibited that mice treated with kefir water showed significant improvement in helper and cytotoxic T-cells levels with a reduction in lung metastasis (Zamberi et al. 2016). Cheng et al. (2014) described the crucial role of commensal bacteria in preserving immune homeostasis by determining the productivity of immune investigation in mucosal tissues. They found that the mice treated particularly with antibiotics were found more susceptible to engrafted B16/F10 melanoma and Lewis lung carcinoma. And this group of mice showed a reduced mean survival time with large tumor foci in lungs. Their further examination exposed that it was because of faulty initiation of the γδT17 (γδT cells

play a significant role in the establishment of the tumor microenvironment and the development of tumor immunity) cell response in the lungs of Abt mice. Supplementation of normal γδT cells or IL17 could restore the weakened immune investigation phenotype in Abt mice model. Overall, the position of commensal bacteria in boosting the host immune system towards cancer was established. On similar lines, in another study, the significance of gut microbiota along with checkpoint inhibitors of immune system was evaluated for lung cancer. Results found that administration of Bifidobacterium cocktail (*B. bifidum, B. longum, B. lactis, and B. breve*) by oral route could control tumor similar to (programmed cell death protein 1 ligand 1) PD-L1 specific antibody therapy (checkpoint blockade). Following both the treatment approaches could almost eliminate the tumor growth. Daillere et al. (2016) referred two bacterial species, *E. hirae* and *Barnesiella intestinihominis* as valuable "oncomicrobiotics" as they improved the efficacy of alkylating immunomodulatory compound cyclophosphamide (CTX) and Th1 cell immune reactions selectively projected longer progression-free survival in both advanced lung and ovarian cancer patients cured with chemotherapy.

Nevertheless the scientific evidence presenting the straight effect of probiotic strains towards lung cancer in humans is incomplete. A study by Serkova et al. (2013) showed that administration of probiotics (*B. subtilis*) to lung cancer patient under chemotherapy could decrease the incidence of gastrointestinal complications by refining their intestinal microflora and prevent the worsening of gut microflora.

10.3.2 Indirect Ways of Probiotic Action in Lung Cancer Therapy

10.3.2.1 Anti-Mutagenic Property and Heavy Metal Detoxification

Benzopyrene, well-known mutagen in the air, widely contribute to the occurrence of lung cancer (Ahmed et al. 2013). Cell suspensions of various species of Bifidobacterium were evaluated towards benzopyrene and the results demonstrated higher anti-mutagenic activities than their cell-free supernatants (Pei-Ren et al. 2002). Yousefi et al. (2019) also showed the ability of live and inactivated probiotic strains to detoxify benzopyrene from aqueous solution. In line with this, the binding ability of nine Bifidobacterium strains to bind with benzopyrene was evaluated recently and results showed the highest ability with *Bifidobacterium lactis* BI-04 HN019 and *Bifidobacterium infantis* BY12 (Shoukat et al. 2019). Also, the capabilities of selected probiotic strains to produce extracellular bioactive compounds with anti-mutagenic properties towards benzopyrene and sodium azide were evaluated in last decade. *B. breve* ATCC 15700 expressed a higher anti-mutagenic effect against sodium azide in the stationary phase but displayed no effect during their exponential growth. *L. sakei* 23K expressed relatively low percent of inhibition of mutagenesis in the exponential phase and no anti-mutagenic activity was noted in the stationary phase. *B. adolescentis* ATCC 15703 showed higher anti-mutagenicity against benzopyrene in the exponential phase. However, it did not possess any anti-mutagenicity against sodium azide in either the exponential or stationary phases. Thus, the authors found that probiotic anti-mutagenic responses

were associated with the bacterial growth phase and mutagen type (Chalova et al. 2008). Anti-mutagenic activity of isolated lactobacilli strains was evaluated in vitro against sodium azide and the highest activity was observed in cell suspensions of 4 strains as compared with their supernatants (Abbas Ahmadi et al. 2014).

The entry of heavy metals and other carcinogens to the human body has been increasing with food chain contamination and this has gained considerable attention in recent times (Nordberg et al. 2011). Scientific reports have established the link between lung cancer and heavy metal and/or carcinogen contamination (Huang et al. 2013). Anti-mutagenic property of probiotics has shown strain-specific by detoxifying toxic metals and carcinogenic compounds, thereby reducing the complications of lung cancer (Monachese et al. 2012; Lili et al. 2018). A mechanism that confers anti-mutagenic property involves the binding of mutagenic compounds to reduce their absorption in the intestine thereby minimizing their retention time and eliminating through feces (Gayathri and Rashmi 2016). In vitro study by Halttunen et al. (2008) demonstrated the heavy metal binding ability of *B. longum, L. rhamnosus, and L. plantarum.* Furthermore, using animal models Gayathri and Rashmi (2016) demonstrated that the enzymatic deconjugation and dehydroxylation of primary bile acids result in the formation of secondary bile acids thereby promoting tumor inhibition ability. Thus, it can be concluded that threat to lung cancer can be abridged using an appropriate combination of probiotic strains which shows a lessening in the entry and continuous elimination of heavy metals from the body (Sharma et al. 2018). On the other hand, as a preventive measure, continuous administration of probiotic bacteria may serve as an option to protect the humans from poisoning by heavy metals, which is identified as one of the major and leading reason for lung cancer (Zoghi et al. 2014).

10.3.2.2 Modulating NK Cells in Host Immune System

Modulating the host immune system is a significant approach by which probiotics advise health benefits. Wide scientific writings are available on how host's irregular immune reaction can be prevented or treated by administrating certain probiotic strains (Konieczna et al. 2012). It is earlier demonstrated that these probiotics exert anti-cancer activity by modulating the immunomodulatory properties on cancer cells (via natural killer (NK) cells, macrophages, T cells), enhancing the production of cytokines, antioxidants, and anti-angiogenic factors and thereby reducing the levels of cancer-specific proteins and pro-carcinogenic enzymes (Dasari et al. 2017). Oral administration of mice with *L. pentosus*, S-PT84 significantly increased NK activity of spleen cells, further leading to the enhanced production of IFN-λ. Enhanced production of IFN-λ was observed due to IL-12 produced by CD11c1 dendritic cells (DCs) after a Toll-like receptor (TLR) 2- and/orTLR4-dependent interaction between DCs and bacteria (Koizumi et al. 2008).

In another study, the immunomodulatory ability of probiotic strains, *L. salivarius* and *L. fermentum*, was evaluated in vitro. The results showed the ability of these strains to modulate both natural and acquired immune responses by activation of NK cells and the expansion of regulatory T cells (Perez-Cano et al. 2010). It was further noted that the mechanism through which these strains exerted anti-cancer functions

is by modulating inflammatory response, helper T cells, and interferon γ levels. Thus, the administration of probiotics to enhance immune function would be beneficial in treating lung cancer.

10.3.2.3 Prevention and Management of Lung Diseases

In recent years, a few clinical trials have demonstrated the beneficial role of probiotics in dropping the incidence of numerous respiratory diseases (Marranzino et al. 2012; Nagalingam et al. 2013). In line with this, probiotic strains of the genus, *Lactobacillus* and *Bifidobacterium*, have revealed the ability to decrease the pathogen load in respiratory system by modulating NK cells and macrophages (Hardy et al. 2013). Earlier to this, two probiotic strains *L. rhamnosus* CRL1501 (Lr05) and CRL 1506 (Lr06), showed inhibition to an intestinal and respiratory pathogen, *Salmonella typhimurium*, and *S. pneumoniae*, respectively. Resistance mechanism of these strains towards the intestinal pathogen was upgraded, but for a lung infection, only *L. rhamnosus* CRL1501strain (Lr05) was found to be more capable to decrease the pathogen load, by increasing IFN-γ (Th1) and IL-4, IL-6, and IL-10 (Th2) cytokine levels in the bronchoalveolar lavage (Salva et al. 2010). IL-17 plays a crucial role in both diagnosis and prognosis of lung cancer (Wu et al. 2016). Oral supplementation of the innovative probiotic mixture has stemmed tumor growth by downregulating IL-17 and its chief producer cells (Li et al. 2016).

Patients with chronic obstructive pulmonary disease (COPD) are at enlarged risk for the development of lung cancer (Houghton 2013). Scientific studies have demonstrated the ability of probiotic strans to treat patients with COPD as they are known to surge the function of NK cells beside with mediators are measured vital in controlling the inflammatory reactions that occur for the duration of COPD exacerbations (Mortaz et al. 2013). Thus, anti-inflammatory approaches using probiotics can be tailored as these two diseases are carefully related to a molecular level (share common activation pathways), and this could be valuable for lung cancer prevention and therapy.

Probiotics administration has explored for preventing and reducing the development of chronic inflammation of airways and lungs in mice models. Scientific evidence suggests that production of reactive oxygen species (ROS) and/or nitrogen species induced by chronic inflammation in the lung may incline persons to lung cancer (Azad et al. 2018). Furthermore, meta-analysis studies have proved the connotation between lung cancer and asthma, suggesting that asthma expressively increases lung cancer risk (Qu et al. 2017). Oral administration of *L. paracasei* L9 attenuates allergic airway reactions in a murine model of asthma by balancing Th1/Th2 responses in the direction of a Th1-dominant state (Wang et al. 2017). Ovalbumin sensitized mice(BALB/c) model was used to evaluate the anti-allergic property of six probiotic strains viz., *B. breve* M-16V, *B. infantis* NumRes251, *B. animalis* NumRes252 and NumRes253, *L. plantarum* NumRes8, and *L. rhamnosus* NumRes6. Results showed *B. breve* M-16V was more effective in reducing acute allergic skin reactions to ovalbumin by reducing the count of eosinophils in bronchoalveolar lavage fluid (BALF) and decreasing the levels of antibodies (both ovalbumin specific IgE and IgG1) and interleukin (IL-4, IL-5, and

IL-10) (Hougee et al. 2010). All these research findings suggest the usefulness of probiotics in prevention of allergy and/or asthma-associated risk of lung cancer.

10.4 Clinical Safety of Probiotics

Gut microbiota shows a major part in the development of cancer that the scientific community is yet to understand clearly. An experiment in cancer therapy investigation lies in defining why certain cancer patients will react to a specific treatment while others with comparable epidemiologic and clinical appearances will not respond. Research findings have recommended that a patient's microbiome may play a better role in reaction to systemic cancer therapy than earlier realized (Hendler and Zhang 2018). Numerous findings in both mouse and human models have explored the role of the microbiome in the prevention of cancer development. Several studies have demonstrated the ability of probiotics both in vitro and in vivo in the prevention of lung cancer (Sharma et al. 2018; Gui et al. 2015). Results have shown anti-tumor response in the growth of tumors and precancerous lesions and the result is not completely consistent across revisions. Thereof, evidences so far have recommended that restoring the function of gut microbiota may have valuable effects in the prevention of cancer and in refining the efficiency and protection of cancer treatment. Furthermore, there are very limited human data evaluating the risk of lung cancer concerning the manipulation of the gut microbiome. While these examinations are far from demonstrating the clinical application of probiotics to prevent lung cancer, further studies may hold great promise.

Eventually, to understand the ability of microbiome-related therapies, the study to be focussed on emerging defined probiotic therapy routines with specific strains and doses that may outcome in a reproducible clinical advantage. Even then, supplementary research is required to regulate the synergistic effect concerning probiotics and anti-cancer drugs that may interpret into enhanced oncologic outcomes (Hendler and Zhang 2018). Despite the debate over the requirement of safety data for probiotics, their cumulative use to treat, prevent, or mitigate lung cancer appears to have caused in a call for similar data from the scientific gathering. So, it is recommended to investigate the clinical safety of probiotics in lung cancer patients because particular patients may be at complex risk for adverse events as they are immunocompromised. On the other hand, in some immuno-compromised patients, reports of occasional circumstances of sepsis succeed probiotics intake (Mehta et al. 2013). If there are any case of adverse events that seems to be produced by the intake of a probiotic strain, particularly it is necessary to verify the uniqueness of organism by molecular testing at recognized laboratory (Doron and Snydman 2015).

10.5 Future Directions of Probiotics for Lung Cancer

There has been an increase in interest in the application of probiotics for their beneficial effects on the host health. Among the various effects, anti-cancer possessions of probiotics have been emphasized in current times (Motevaseli et al.

2017). This is mainly due to the increasing evidence of the interaction of gut microbiota and pathophysiological processes of disease within the human host (Day et al. 2019). Scientific evidence suggests that these commensal microorganisms bring out such effect by destruction of the microbiota growth occupied in the building of mutagens and carcinogens, modification in carcinogen metabolism, and defense of DNA from oxidative damage as well as instruction of immune system (Motevaseli et al. 2017). In brief, recent findings on assessment of the effect of probiotics on lung cancer have reinforced their favorable effects both in vitro and in vivo. However, pre-clinical or clinical evaluations are not sufficient to adopt about their usage. Even though, substantial progress has been made in extensive analysis of gut commensal microbiota and its result on the balance of pro- and anti-inflammatory forces of the immune system. Many queries remain unanswered on which immune cells are the vital targets for such probiotic activities. In future, it is more imperative to regulate the biological mechanisms and physiological interactions behind the scheduled probiotic action on lung cancer (Tian et al. 2019). Once these biological mechanisms and physiological interactions are validated and defined confidently, the transformation from laboratory science to clinical interventions using probiotics could be accelerated substantially (Day et al. 2019) for the use of probiotics to treat and prevent lung cancer.

10.5.1 Combined Chemotherapy Strategy with Probiotics for the Management of Lung Cancer

Quite a few of clinical trials have emphasized the efficacy of probiotics administration to cancer patients receiving chemotherapy, and their results have demonstrated the probiotic ability to reduce gut-related side effects (Vivarelli et al. 2019). Brain inflammation altering cognitive behavior and gut microbiota imbalance are the combined events that occur in the context of chemotherapy. The link between altered gut microbiota and behavioral deficits via activation of the central and peripheral immune system that are implicated with chemotherapy is now validated using mice model (Loman et al. 2019). However, the scientific evidence on the administration of probiotics to overcome these effects has not been demonstrated at the clinical levels. Furthermore, diarrhea is the most common undesirable side effect related to chemotherapy treatment (Wei et al. 2018). Lu et al. (2019) systematically assessed the effectiveness of probiotics in preventing chemotherapy-induced diarrhea among patients with malignant tumors. In specific, Tian et al. (2019) investigated the role of *Clostridium butyricum* administration to lung cancer patients undergoing chemotherapy. The result demonstrated that *C. butyricum* could reduce chemotherapy-induced diarrhea in lung cancer patients and reduce systemic inflammatory responses. Thus, these evidence-based indications signify the use of probiotics for treating chemotherapy-induced diarrhea.

10.6 Conclusion

Probiotics have gained increased medical attention in recent times owing to their favorable properties on the host health. Administration of probiotics by oral route is known to impart multiple effects on hosts viz., expansion of the gastrointestinal barrier, normalization of the intestinal microflora, and inhibition of pathogens or carcinogenesis in the gut. Probiotics play a share in dropping the risk of cancer by improving immune system or/and anti-inflammatory activities (Nazir et al. 2018). Furthermore, the beneficial effects of probiotics in the prevention and treatment of lung cancer are still controversial due to the fact of application of animal models without further validation with clinical trials and limited data on clinical safety of probiotics. Since there is a significant difference in metabolism in animal models and the human body, it is difficult to obtain satisfactory results. Therefore, long-term human complementary readings are recommended to address the contention (Nazir et al. 2018). Further translational and clinical research in humans should be necessary to inspect the possibility of deploying the gut microbiota to expand outcomes in lung cancer (Hendler and Zhang 2018).

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Bacteriocins of Probiotics as Potent Anticancer Agents

11

Pallvi Sharma and Santosh Kumar Tiwari

Abstract

Cancer is one of the momentous causes of death worldwide despite the availability of advance detection and treatment methods. The present chemotherapeutic treatments are nonspecific and toxic to human cells. Thus, there is demand for safe and effective drugs for the treatment of various types of cancer. There are various studies reporting the potential of bacteriocins against cancer cells leaving healthy cells unaffected. Bacteriocins are ribosomally synthesized small antimicrobial peptides that generally inhibit the related strains of bacteria. Few bacteriocins of probiotic lactic acid bacteria are safe for human consumption and have got GRAS (generally regarded as safe) status. This chapter highlights the recent developments on applications of bacteriocins of probiotics along with other bacteriocins as anticancer agent, their cytotoxicity, efficacy, and mode of action against cancer cells. The emphasis has been given for search of effective and safe bacteriocins as alternative to clinical therapeutics for the treatment of cancer.

Keywords

Probiotics · Bacteriocins · Mode of action · Cytotoxicity · Nisin · Pediocin · Plantaricin

P. Sharma · S. K. Tiwari (✉)
Department of Genetics, Maharshi Dayanand University, Rohtak, Haryana, India
e-mail: santoshgenetics@mdurohtak.ac.in

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11.1 Introduction

Nowadays, cancer is one of the most important causes of increasing mortality and morbidity. Recently, approximately 9.6 million cancer mortality was estimated worldwide (Silas et al. 2019). Cancer is manifested due to uncontrolled cell differentiation, proliferation, and growth of cells. Cancer cells may develop an abnormal potential to divide due to exposure to toxic compounds such as benzene, asbestos, aflatoxin, vinyl, etc., and ionizing radiation, i.e., radon, UV, X-ray, uranium, etc. There are several pathogenic and genetic factors responsible for cancer (Kaur and Kaur 2015). Normally, cells have regulating mechanism, so that there is a balance maintained between the number of cells formed and the cell death. But in the case of cancer cells, the number of a particular cell does not remains constant because cancer cell stops responding to regulatory mechanism of cell division and leads to uncontrolled cell division and growth. General characteristics of cancer cells involve unresponsiveness to regulatory signals, i.e., inhibitory signals, escape apoptosis, show self-sufficiency in growth signals, sustained angiogenesis, have great replicative potential, and may show metastasis (Kaur and Kaur 2015).

Generally, the common treatment strategies for cancer involve surgical resection of tumors followed by radiation and chemotherapy. The chemotherapy does not specifically target cancer cells rather may also involve drug-induced damage to healthy cells. The major problem is the resistance to chemotherapy in cancer cells due to factors like increasing DNA repair mechanism, increased expression of drug transporter, and detoxifying agents like enzymes. Therefore, there is an urgent need to find novel treatment strategies that can specifically target cancer cells without affecting healthy cells. Various live bacteria and their purified products including bacterial toxins, peptides (e.g., bacteriocins), proteins, and enzymes have anticancer potential and can be used as new anticancer agents (Nguyen and Nguyen 2016).

Bacteriocins are heat stable, ribosomally synthesized antimicrobial peptides produced by most groups of bacteria to inhibit the growth of closely related species. These bacteriocins can be used as potential anticancer drug showing selectivity against cancer cell as compared to normal cells. These peptides may be applied either directly to inhibit cancerous cells or inhibition of bacteria associated with the initiation of the cancer. Nisin produced by several strains of *Lactococcus lactis* is one of the best-studied bacteriocin available commercially (Kumariya et al. 2019). Treatment of cancer cells with increasing concentrations of nisin-induced DNA fragmentation and apoptosis in different cancer cell lines has been reported (Moll et al. 1999).

11.2 Bacteriocins

Similar to virtually all bacteria, LAB probiotics also produce ribosomally synthesized substances of proteinaceous nature with antimicrobial activity. These substances are collectively called bacteriocins (Alvarez-Sieiro et al. 2016). Many bacteriocins have received special attention due to their potential as food

preservatives and therapeutic antimicrobials. Attractive characteristics of many bacteriocins are their thermostability and activity over a broad temperature and pH range. Furthermore, they are nonimmunogenic and are generally colorless, odorless, and tasteless, all of which makes them particularly attractive for health care applications (Alvarez-Sieiro et al. 2016). Because of the widespread emergence of resistance to most of the commonly used therapeutic antibiotics (Woolhouse et al. 2016), new classes of antimicrobial agents are desperately being sought. In order to reduce the use of antibiotics in clinical settings and in agriculture, an attractive potential substitute could be the use of probiotics and/or their bacteriocins. Tagg et al. (1976) defined bacteriocins as being largely active against closely related bacteria; however, it has now also been established that many have some activity against more distantly related bacteria (Gupta et al. 2016). The sensitivity of a target bacterium to bacteriocins depends on the physicochemical environment of the interaction, with factors such as the pH, ionic strength, and presence of neutralizing or membrane-disrupting molecules being especially pertinent (Belguesmia et al. 2010). Thus, the antimicrobial nature of bacteriocins has been established and widely studied. The anticancer properties of these bacteriocins have been least explored and need attention for their applications in the treatment of deadly diseases, such as cancer.

Bacteriocins have been divided in to four classes on the basis of their genetic, biochemical, and structural characteristics -

11.2.1 Class I

These bacteriocins are small 19–50 amino acids, are heat stable, and have posttranslational modifications of peptides. These modified peptides are resulted with nonstandard amino acids such as lanthionine, dehydrobutyrine, labyrinthine, β-methyllathionine, and dehydrolanine. Dehydrobutyrine and dehydroalanine are α-β unsaturated amino acids, which most commonly occur in the structure of these bacteriocins. Class I bacteriocins form pores and intracellular rings and generally have broad-spectrum activity (Kumariya et al. 2019). The class I has further three subclasses:

Class Ia includes lantibiotics that are relatively elongated flexible cationic and pore-forming peptides. These are <5 kDa biologically active peptides produced by wide varieties of Gram-positive bacteria. These are antibiotic peptides containing lanthionine (Lan), dehydrated residues, and β-methyl lanthionine (MeLan). Nisin is the most studied bacteriocin of this group (Klaenhammer 1993).

Class Ib includes labyrinthopeptins that are globular, compact, and either negative or neutral peptides. They have enzyme inhibitors and are immunologically active and carbacyclic antibiotics containing labyrinthin and labionin (Klaenhammer 1993), e.g., labyrinthopeptins and A1.

Class Ic includes sactibiotics that are antibiotics containing sulfur to alpha carbon, e.g., thuricin CD (Klaenhammer 1993).

11.2.2 Class II

These bacteriocins are low-molecular weight (<10 kDa) peptides, membrane active, heat stable up to 100 °C, and posttranscriptionally unmodified and show narrow-spectrum activity. These are non-lanthionine peptides and generally interact with anionic lipids present in the membranes of Gram-positive bacteria (Cui et al. 2012). This class is further subdivided into four subclasses (Klaenhammer 1993) and is detailed as follows.

Class IIa bacteriocins are usually small heat stable peptides and synthesized as precursor. These peptides further undergo double glycine residue processing and become an active peptide having a consensus sequence of YGNGV-C on N-terminal end, e.g., pediocin PA-1, leucocin A, sakacin P, and sakacin A (Fimland et al. 2005).

Class IIb bacteriocins are comprised of two component systems that require two different peptides for their activity. They are generally known for pore formation, e.g., lactococcins G, plantaricin JK, and plantaricin EF (Heddle et al. 2001).

Class IIc bacteriocins are secondary dependent, small unclassified, circular, non-lanthionine, and heat stable, e.g., enterocin AS-48, gassericin A, and garvicin ML (Ibrahim 2019).

Class IId—These bacteriocins are unmodified, without leader sequence, linear, and non-pediocin-like peptides, e.g., aureocin A53 and bactofencin A (Ibrahim 2019).

11.2.3 Class III

These bacteriocins are large in size with molecular masses of >30 kDa, are heat labile, and have narrow spectrum of inhibitory activity. These are unmodified bacteriocins that show bacteriolytic and nonlytic activity against target bacteria. They cause membrane permeabilization by pore formation, e.g., helveticin M, helveticin J, and enterolysin A (Klaenhammer 1993).

11.2.4 Class IV

These bacteriocins are circular and heat-stable molecules and have large proteins, i.e., a mixture of lipids and carbohydrates (hippe et al. 2015), e.g., sublancin and glycocin F (Ibrahim 2019).

11.3 Anticancer Property of Bacteriocins

Bacteriocins are mostly cationic in nature due to the presence of a large number of lysine and arginine amino acids. The cationic nature of bacteriocins plays an important role during interaction with the anionic lipid content of cell membrane of target bacteria. Bacteriocins are involved in depolarization of membrane and also

lead to membrane lytic effects. They insert their amphipathic peptide in cell membrane and lead to the formation of pores in membrane. Due to cationic nature, bacteriocins also interact with negatively charged cell membrane of cancer cells. Such interaction facilitates the formation of pores in the membrane leading to cell death (Van Horssen et al. 2006).

The other unique characteristic of bacteriocins is their amphipathic nature having unique transmembrane conformation with external hydrophilic and internal hydrophobic components. Bacteriocins show minimal cytotoxicity against human cells, and therefore, it is usually considered as generally regarded as safe (GRAS) molecule (Van Horssen et al. 2006). Hydrophobic nature of bacteriocins has major advantage as anticancer agent, and this interaction is stronger than Van der Waals interaction that plays a crucial role in antitumor property of a bacteriocin. These antimicrobial peptides lead to the production of tumor necrosis factor alpha (TNF-α) that is involved in a number of effects such as apoptosis, necrosis, cell migration, differentiation, etc. The production of TNF-α and a number of cytokines leads to the activation of various signal transduction pathways like TNFR-1 signaling pathway with outcomes like apoptosis, cell proliferation, etc.

Few bacteriocins may alter the angiogenesis process of cancer cells (Huang et al. 2020). Angiogenesis, new blood vessels formation, is the main process that aggravates cancer cell proliferation and provides nutrients and oxygen to tumor cells. The cancer cell proliferation may be controlled by inhibiting angiogenesis, i.e., by starving cancer cells. There are various drugs used for inhibition of angiogenesis, e.g., axitinib (Inlyta) for kidney cancer, bevacizumab, everolimus, sorafenib, etc. Along with their antiangiogenic effect, they also have prevalent side effects like blood pressure increase, diarrhea, delayed wound healing, heart failure, etc. Thus, there is a crucial need for search of potent agent that inhibits angiogenesis of cancer cells. The targeted activity of bacteriocins against cancer cells without affecting healthy cells makes them more suitable for cancer treatment (Dobrzyńska et al. 2005).

11.4 Mechanism of Action of Bacteriocins Against Cancer Cells

Cancer cells have net negative charge on their membrane surface, which facilitates their interaction with positively charged bacteriocins. The negative charge on cancer cell surface is mainly due to the presence of high-level anionic phosphatidylserine, heparin sulfates, O-glycosylated mucins, and glycosylated gangliosides (Baindara et al. 2018). The phospholipid distribution in healthy cells is asymmetric, whereas cancer cells loose the asymmetry. Due to asymmetric distribution of phospholipids on both the leaflets of membranes of healthy cells, they have net neutral charge on their surface. Cancer cells exhibit an overexpression of O-glycosylated mucin (Taraboletti et al. 1989) and phosphatidylserine on the outer leaflet of membrane surface and impart net negative charge. The negative charge exhibits an electrostatic interaction with the positively charged bacteriocins (Baindara et al. 2018).

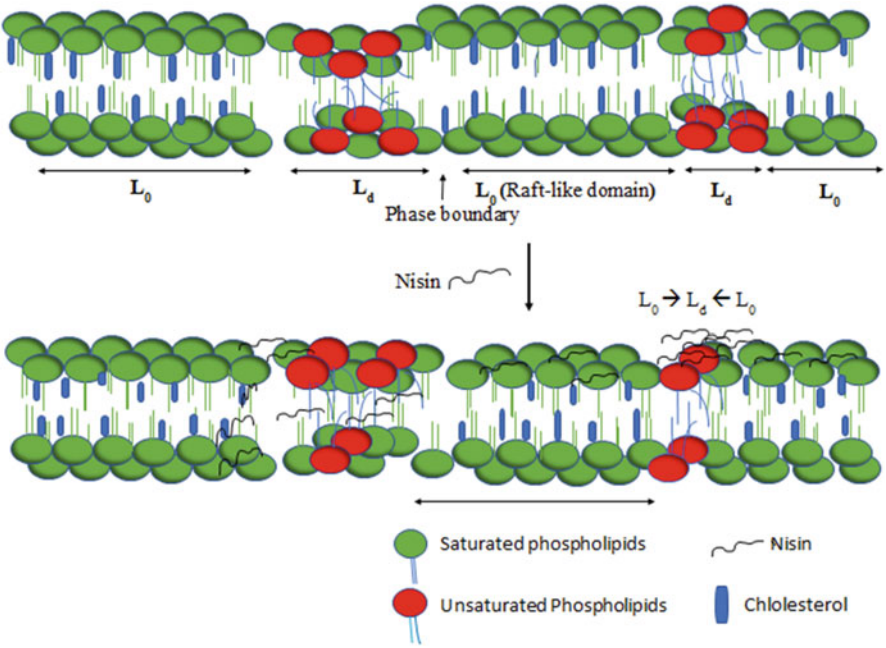

Fig. 11.1 Nisin interaction with neuroblastoma cells showing fusion of raft-like domains. (Prince et al. 2019)

Fluidity of membrane plays a crucial role in induction of apoptosis in cell leading to cell death. Recently, it has been found that various anticancer agents involve in activation of apoptosis through altering the fluidity of cancer cell membrane. Aggregation of death receptors like DR4, DR5, FAS, etc., and alteration of lipid raft composition in cell membrane may alter the fluidity of membrane. Change in membrane fluidity leads to activation of various signaling pathways (Thomas and Delves-Broughton 2005). Nisin interaction with neuroblastoma cell membrane intensifies the fluidization of membrane (Fig. 11.1) through ROS generation, Ca^{2+} influx, and apoptosis results remarkable loss of neuroblastoma cell growth. Nisin interaction undermines the raft-like regions (Lo) through disrupting the structural integrity of nonraft-like regions (Ld), in turn interrupting the signaling pathway of proliferation of neuroblastoma cell (Prince et al. 2019). Increased membrane fluidity facilitates interaction with anticancer peptides (ACPs) and ultimately leads to deterioration of cancer cell membrane (Baindara et al. 2018). The other important characteristic of cancer cells is increased surface area due to the presence of a number of microvilli. This facilitates more chance of interaction of bacteriocin with surface and also enables anticancer peptide (ACP)-mediated cytotoxicity killing of selective cancer cell without affecting the healthy cells. An increased amount of anionic lipid cardiolipin in membrane of mitochondrial makes it negatively charged. This further facilitates ACPs such as bacteriocins and leads to the distribution of

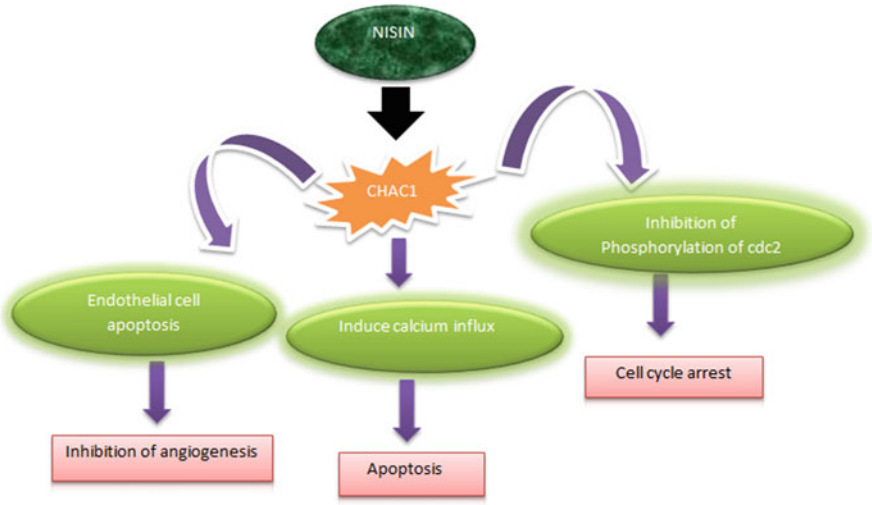

Fig. 11.2 Mechanism of action of nisin in tumor cells (Joo et al. 2012; Varas et al. 2020)

mitochondrial membrane integrity. This disruption may result in release of content like cytochrome C, which induces or activates the cell death pathway.

Bacteriocins modulate cancer cell proliferation and induce apoptosis in tumor cells. Nisin Z, a variant of nisin, induces cell death through different ways in cancerous cell, i.e., by destructions of cell membrane, altering gene expression of cell signaling and depolarizing mitochondrial membrane. (Lewies et al. 2018). Nisin induces apoptosis in tumor cell through expression of ChaC glutathione-specific gamma-glutamylcyclotransferase 1 (CHAC1) gene, which is a proapoptotic cation transporter regulator and is also known as apoptotic mediator (Fig. 11.2). Induction of apoptosis through CHAC1 may enhance calcium ion influx and also promote cell cycle arrest, thus decreasing cell proliferation (Varas et al. 2020; Joo et al. 2012).

There are various bacteriocins, e.g., pyocins, nisin, azurin, colicin, pediocin, microcin, etc., that have shown antineoplastic properties against cancer cell lines. These bacteriocins may directly inhibit endothelium cell proliferation through p53 or may inhibit motility and migration of endothelial cells. In tumor cells, there is an overexpression of several growth factors like VEFG (vascular endothelial growth factor) and bFGF (fibroblast growth factor). Bacteriocins influence angiogenesis process either by interacting with growth factors or by interacting with their receptors. For example, a variant of bacteriocin azurin enters into HUVEC (human umbilical vascular endothelial cell) and inhibits migration of HUVEC. It may also inhibit capillary tube formation by inhibiting phospho-VEGFR-PI2K signaling pathway. It also alters distribution of cell mobility and migration-associated proteins such as F-actin, P-axillin, and FAK (Mehta et al. 2011).

11.5 Bacteriocins Investigated as Potential Anticancer Agents

There are several bacteriocins that have been reported to show anticancer activity against different cell lines. Few selected bacteriocins showing potential application as anticancer agents have been depicted in Table 11.1 and are described as follows.

11.5.1 Nisin

Nisin is a 3.49-kDa heat-stable, pentacyclic peptide secreted by different strains of *Lactococcus lactis*. After posttranslational modification, molecular structure of each nisin consists of lanthionine (Lan), 1-didehydroaminobutyric acid (Dhb), 4-methyllanthionine (MeLan), and 2-didehydroalanine (Dha) amino acid molecules. Nisin A, nisin Q, and nisin Z are produced by *Lactococcus lactis,* but nisin U is exceptionally produced by *Streptococcus uberis*. There have been 8 different types of nisin variants, which are known namely as nisin A, nisin Q, nisin Z, nisin U, nisin F, nisin B, nisin H, and nisin U2 (Lubelski et al. 2008).

Nisin makes wedge-like pore in the target cells by interacting with lipid II moiety present in the membrane. It shows cytotoxicity against HepG2 and MCF-7 (human breast adenocarcinoma cell line). These cell lines exhibit 112.25 and 105.46 μM, respectively for IC_{50} value for HT29 and Caco-2 cell line (human carcinoma of colon and colorectum) is 89.9 μM and 115 μM, respectively. Nisin have an apoptosis stimulation effect on colon cancer line induced through intrinsic pathways and lead to the stimulation of programmed cell death of cancerous cells (Norouzi et al. 2018). Nisin alters the apoptotic index of cancer cells by altering the expression of bax and bcl-2 genes involved in intrinsic apoptotic pathway in colorectal cancer cells. Nisin increases the bax/bcl-2 ratio in colorectal cancer cell protein and mRNA (i.e., upregulation of Bax and downregulation of Bcl-2), which induces activation of apoptosis process. Nisin lowers the cell proliferation and metastasis potential in SW48, HT29, and Caco-2 cell line by downregulating some important molecular biomarker genes, i.e., CEA, MMP2F, CEAM6, and MMP9F genes (Kamarajan et al. 2015).

Nisin shows antiproliferatory and antimetastatic potential in HNSCC tumorigenesis by inducing apoptosis, reducing cell proliferation and stimulating the cell cycle arrest. The apoptotic effect is due to upregulatory CHAC1 expression, a proapopototic cation transport regulator also known as an apoptotic mediator by stimulating extracellular influx of calcium ion. It preferentially interacts with phosphotidyl choline, disturbing the phospholipid organization of membrane, thus allows an influx of ions. Nisin reduces the proliferation in HNSCC cells by arresting cell in G2 phase of cell cycle (Joo et al. 2012). Nisin ZP, 3.47 KDa, a natural variant of nisin induces apoptosis in HNSCC cells at high level as compared to nisin (low content). Nisin ZP enhances the endothelial cell apoptosis and inhibits angiogenic sprouting through CHAC1. Nisin ZP induces apoptosis through calpin-dependent pathway in HNSCC. It also induces apoptosis in HUVEC (human umbilical vein endothelial cell) dose dependently, by decreasing intratumoral microvessel density

Table 11.1 Few important bacteriocins and their activity against cancer cells

S. no.	Bacteriocins	Producer bacteria	Anticancer activity	References
1	Nisin	*Lactococcus lactis*	Cytotoxicity against HepG2 and MCF-7 cell line Alters the apoptotic index of cancer cell by altering the expression of bax and bcl-2 genes Lowers the cell proliferation and metastasis potential in SW48, HT29, and caco-2 cell lines Interacts with phosphotidyl choline allowing an influx of ions and thus reduces the proliferation in HNSC cells	Joo et al. (2012), Shin et al. (2016), Norouzi et al. (2018), Kamarajan et al. (2015), Ahmadi et al. (2017)
2	Plantaricin	*Lactobacillus plantarum*	Binds to lysine and aspartate residues in histidine kinase through electrostatic interactions Affects the cells by membrane permeabilization and leads them to apoptosis with necrosis.	Nakayama et al. (2000), Sand et al. (2013)
3	Pyocins	*Pseudomonas aeruginosa*	Pyocin S2 shows cytotoxic activator against AS-II, mKS-ATU 7, HeLa, and HepG2 cell lines Also shows inhibitory effect on HepG2 and Im9 without affecting normal cell line of HFFF (human fetal foreskin fibroblast)	Nakayama et al. (2000), Sand et al. (2013), Abdi-Ali et al. (2004)
4	Pediocin	*Pediococcus* spp.	Pediocin PA-1 shows anticancer effect against A-549 (human lung carcinoma cell line) and DLD-1 (human colon adenocarcinoma cell line) Pediocin CP2 and its variant show cytotoxic effect against HepG2, MCF7, and HeLa cell line	Kaur and Kaur (2015), Rodrigues et al. (2019)
5	Colicin	*E. coli*	Colicin A preferentially inhibits the HS913T fibrosarcoma cell lines Colicin E1-E5 and K show cytotoxic activity against V79 (hamster fibroblast) cell line Colicin A, E, and E3 involve in cell cycle alteration in MRC5 (human fibroblast cell line) and MCF7 (human breast cancer cell line) osteosarcoma cell line HOS, fibro-sarcoma cell line HS913T, and MDA-MD-231 cell line Colicin E1 and E3 cause necrosis in oncogene V-myb-transformed chicken monoblast Colicins either interact with the G1 phase of cell cycle thus interfering with the cell proliferation or may disrupt the organization and potential of plasma membrane	Varas et al. (2020), Cascales et al. (2007), Punj et al. (2004)

(continued)

Table 11.1 (continued)

S. no.	Bacteriocins	Producer bacteria	Anticancer activity	References
6	Microcin E492	*Klebsiella pneumoniae*	It shows cytotoxicity against Jurkat (T-cell derived from acute T cell leukemia), Hela (human cervical adenocarcinoma), RJ2.25 (a variant of Burkett's lymphoma), and colorectal carcinoma cells Causes DNA fragmentation, membrane potential disruption, calcium ion release, and exposure of phosphatidylserine on outer surface followed by programmed cell death, i.e., apoptosis	Lagos et al. (2009)
7	Duramycin	*Streptoverticillium cinnamoneus*	Interacts with target cells leading to imbalance in plasma membrane and influences ion transportation, which is facilitated by pore formation on cell membrane Shows inhibitory effect on ATPase activity and increased permeability and blockage of Na^+-K^+-ATPase on cell membrane	Silas et al. (2019), Tagg et al. (1976), Taraboletti et al. (1989), Rodrigues et al. (2019), Nakamura and Racker (1984), Oliynyk et al. (2010)
8	Bovicin	*Streptococcus bovis* HC5	Decreased the cell viabilities of MCF 7 and $HepG_2$ human cell line	Kaur and Kaur (2015), Mantovani et al. (2002), Chumchalová and Šmarda (2003)

(Cascales et al. 2007). Nisin along with doxorubicin, a chemotherapeutic agent for cancer, shows synergistic antitumor effects that involve chromatin condensation and nuclear material marginalization (Kaur and Kaur 2015).

11.5.2 Plantaricins

Different strains of *Lactobacillus plantarum* produce plantaricin, which are low molecular mass peptides (~2.4 kDa). Plantaricins have amphiphilic nature to facilitate the membrane channel formation. It binds with negatively charged membrane and makes a strong connection with glycolate protein of membrane shown by microfluorometric technique. Plantaricin A, E, F, J and K show activity against *Staphylococcus epidermidis* by rapid lysis and repression of growth of bacterial cell (Michel-Briand and Baysse 2002).

It also acts as a pheromone that has a new interaction mechanism with membrane in which they make α-helical structure induced by membrane and lead the peptide to interact with particular receptors. Plantaricin initially binds to one or more lysine and aspartate residues (interphase positioned) in histidine kinase through electrostatic interactions that enable chiral interactions between plantaricin and histidine kinase (Nakayama et al. 2000). It affects normal human B- and T-cells, Reh cells, and Jurkat cells. Reh cells are from human B-cell leukemia and Jurkat cells from human T-cell leukemia. All four cells were tested with plantaricin affecting membrane permeabilization and lead them to apoptosis with necrosis (Baindara et al. 2017).

11.5.3 Pediocins

The precursor of pediocin is a prepeptide of 62 amino acids, which undergoes cleavage through ABC transporter system, resulting in the formation of mature peptide. A number of pediocin have been identified, namely pediocin F, CP-2, AcH, K1, and L50 (Baindara et al. 2017). Pediocins are sensitive to proteases, e.g., pepsin, protease, papain, etc. Pediocin PA-1 shows anticancer effect against A-549 (human lung carcinoma cell line) and DLD-1 (human colon adenocarcinoma cell line). The exact mechanism of action of pediocin is still unknown. The N-terminal region of pediocin consists of conserved Y-G-N-G-V/2 "Pediocin Box" motif and two cysteine residues, resulting in the formation of disulfide bridge. Through these disulfide bonds, N-terminal region of peptide gets folded, which helps in the binding with the membrane. The C-terminal region forms a hairpin-like domain and mediates leakage in the hydrophobic region of target membrane. Pediocin CP2, produced by *Pediococcus acidilactici* CP2 MTCC51101 and its variant, shows cytotoxic effect against HepG2, MCF7, and HeLa cell line. It was observed when MCF-7, HepG2, and CP2 cell lines treated with pediocin either native or rec-pediocin, they retained 10.74, 5.52, 2.133 percentage of viabilities, respectively. Human colorectal adenocarcinoma and human lung carcinoma cell line

growth were inhibited by pediocin PA-1 isolated from *P. acidilactici* PAC1.0 (Kaur and Kaur 2015).

11.5.4 Colicins

Usually, colicins are plasmid-encoded, high-molecular weight bacteriocins, i.e., more than 20 kDa, secreted by generally *E. coli* and other related species. It was identified in 1925 in *E. coli* culture by Gratia. In 1946, Fredericq and Gratia coined the term colicin. They also displayed proteinaceous nature of colicin and their activity spectra. According to activity and mechanism of action, there are 30 different types of colicins that have been identified (Tomita et al. 2000). Colicins are SOS regulated and produced in response to stress such as oxygen depletion and nutrient deficiency.

The mechanism of action of colicin involves binding to target membrane, translocation through the membrane, and finally the cell death. Usually colicin binds on outer membrane and then interacts with Ton or Tol complex of membrane protein killing the cells either by perforation (such as in colicin IA, IB, A, N, B, E, L, U, K, 5, and 10), DNAase activity (colicin E2, E8, E7, and E9), or 16sr RNA or tRNA cleaving and resulting in protein synthesis inhibition (Colicins E6, E4, E5, E3, and D) (Harkness and Braun 1989). There have been 17 colicins identified and studied in detail, which are colicin 17, A, D, E1, E3, E4, E2, E5, E7, E8, E6, E9, IB, IA, K, N, L, and M. All bacterial cells secreting colicins protect themselves from their cytotoxic effect by synthesizing immunity proteins. Mechanism of action of colicin involves pore formation, endonuclease activity, and some other mechanisms like inhibition of murine biosynthesis. They lead to the formation of pore in cytoplasmic membrane, thus disrupting their electrochemical potential. Colicin forms a helical hairpin into lipid bilayer of membrane after insertion of eight amphiphilic and two hydrophobic colicin helicals (HA and H9). Colicin M uniquely inhibits biosynthesis of murine and lipopolysaccharide O-antigen, which leads to killing of cells (Kohoutova et al. 2014).

Colicins show anticancer activity in different cancer cell line in vitro like colon cancer, bone cancer, and breast cancer cell lines. Colicin A preferentially inhibits the HS913T fibrosarcoma cell lines. Colicin E1-E5 and K cytotoxic activity have been identified against V79 (hamster fibroblast) cell line. Colicin A, E, and E3 involve in cell cycle alteration in MRC5 (human fibroblast cell line), MCF7 (human breast cancer cell line), osteosarcoma cell line HOS, fibro-sarcoma cell line HS913T, and MDA-MD-231 cell line. Colicin E3 shows cytocidal effect against Hela cell. Colicin A and E2 lead to dose dependent decrease in viability of murine lymphoma cell line. Colicin E1 and E3 cause necrosis in oncogene V-myb-transformed chicken monoblast (Tomita et al. 2000). Colicin when injected intradermally into tumor, decreases the volume of tumor. Colicin E3 and U are not involved in any considerable change in cell cycle (Chumchalová and Šmarda 2003). Colicin E3 displays more prominent cytocidal effect on uterine carcinoma cell line. There has been found a significant increase in the survival of LP-2 plasmacytoma-transplanted mice when

treated with colicin A. Colicins either interact with the G1 phase of cell cycle, thus interfering with the cell proliferation or may disrupt the organization and potential of plasma membrane (Punj et al. 2004).

11.5.5 Azurin

It is about 14 kDa copper containing antimicrobial peptide. It is a member of cupredoxin family and produced by *Pseudomonas aeruginosa*. Azurin displays basic conserved structure in which two main β-sheets form a rigid β-sandwich (Nguyen and Nguyen 2016). This peptide selectively penetrates different cancer cells such as breast cancer (MCF-7), osteosarcoma (U20S), and melanoma (UISU-mel2) and did not affect healthy cells (Kwan et al. 2009). The p28, a variant of azurin, penetrates UISO-mel2, mel6, and HUVEC cell line. It displays inhibition of HUVEC migration and inhibition of capillary tube formation. It is also involved in alteration of distribution of migration-associated proteins and cell motility. Azurin penetrates human cancer cell, melanoma UISO-mel2, and interferes with tumor suppressor gene p53 and induces apoptosis. Azurin is internalized and predominantly present in cytosol. Its intracellular trafficking to nucleus is p53 dependent. This may also influence the stimulation of apoptotic factor such as Bax in mitochondria, which significantly increases the release of cytochrome C, thus induces apoptosis. Azurin p28 shows selective translocation and cytotoxic effect against chronic and acute myeloid leukemia cell line through interfering with angiogenesis and by inducing apoptosis in HUVEC (Mehta et al. 2011; Lagos et al. 2009).

11.5.6 Microcins

These are low-molecular weight bacteriocins (<10 kDa) produced by several enterobacteria under stress conditions. Microcin E492 is secreted by *Klebsiella pneumoniae* and has a molecular mass of about 7 kDa. It imparts a considerable antitumor activity through disrupting the membrane potential by forming pore in the cell membrane (Kristiansen et al. 2005). It also displays DNA gyrase activity like other antineoplastic drugs. Microcin E492 induces apoptosis by regulating calcium ion influx from intracellular stuffs and induces ion channel formation (Cascales et al. 2007). It shows cytotoxicity against a number of malignant cell lines such as Jurkat (T-cell derived from acute T-cell leukemia), Hela (human cervical adenocarcinoma), RJ2.25 (a variant of Burkett's lymphoma), and colorectal carcinoma cells. Cancer cells treated with microcin-E492 show a number of changes such as DNA fragmentation, activation of caspases, mitochondrial membrane potential disruption, calcium ion release, cell shrinkage, and exposure of phosphatidylserine on outer surface, followed by programmed cell death, i.e., apoptosis (Kristiansen et al. 2005).

11.5.7 Pyocins

There are plasmid encoded, highly stable, cationic, and small antimicrobial proteins produced by *Pseudomonas aeruginosa* species (mostly) and some other lactic acid-producing bacteria (Shah et al. 2013). A number of pyocins have been identified including P-2, SJ-1, K1, F, L-50, L, AcH, ACM, etc. Synthesis of pyocin is under control of a number of housekeeping genes of recA and prt gene family. Basic structure of pyocin consists of two components: the larger display bacteriocidal activity and the smaller one produces immunity proteins. The large component further consists of different structural domains, N-terminally located receptor-binding domain, central translocation domain, and C-terminally located DNAase domain (Abdi-Ali et al. 2004). Pyocins are further divided into 3 types:

1. R type—Nuclease and protease resistant involves in disrupting membrane potential by permeabilization (Sand et al. 2013).
2. F type—They are flexible and structurally similar to tails of phages and having rod-like, noncontractile structure (Sand et al. 2013).
3. S type—These are nuclease and protease sensitive having two components. The larger component of S type pyocin carries DNAase activity (S1, S3, S2, and AP41), tRNA activity (S4), and channel-forming activity (pyocin S5). The smaller component carries immunity proteins. (Michel-Briand and Baysse 2002). The pyocin S2 shows cytotoxic activator against AS-II, mKS-ATU7, HeLa, HepG2 (human hepatocellular carcinoma), and ImG (human immunoglobulin secreting cell line of myloma) (Sand et al. 2013).

Pyocins show inhibitory effect on different tumor cell lines such as HepG2 and Im9 and have no effect on normal cell line of HFFF (human fetal foreskin fibroblast). It also shows lethality against L60T mice fibroblast cell line. Im9 Epstein–Barr transformed lymphoblasts are more sensitive to pyocin S2 as compared to hepatocellular carcinoma (HepG2) cell line. Binding of pyocins to the mammalian cell is facilitated by iron-related receptors such as transferring receptors (Iwamoto et al. 2007).

11.5.8 Duramycin

Streptomycetes produce an antibiotics known as duramycin. It has tetracyclic antimicrobial peptide, is ribosomally synthesized, and shows posttranslational modifications. Duramycin has 19 amino acids residues of molecular mass of ~ 2 kDa (Rodrigues et al. 2019). The posttranslational changes of duramycin consist of enzymatic addition of three thioether bonds. This binding increases the proteolytic stability and provides selectivity to phosphatidyletanolamine (PE), which is presented on cell membrane of Gram-positive and Gram-negative bacteria (Bennik et al. 1997).

Duramycin interacts with target cells leading to imbalance in membrane integrity facilitated by pore formation on cell membrane (Oliynyk et al. 2010). Cellular plasma membrane of Ehrlich tumor cells is affected by duramycin and shows inhibitory effect on ATPase activity, increasing permeability and blockage of Na^+-K^+-ATPase on cell membrane (Nakamura and Racker 1984). All these properties show that duramycin has antineoplastic activity. Phophatidyl ethanolamine (PE) is present on surface of cancer cells leading to decrease in the tumor cell proliferation and increase in apoptosis. Cell death and release of Ca^+ depending on time and concentration of duramycin were observed on cancer cell lines (CaCo-2, AsPC-1, MDA-MB-231, LOVO, Colo 320, HCT 116, MIA PaCa-2, JJN 3, MM.IS, and U266B1) (Bennik et al. 1997).

11.5.9 Bovicin

Bovicin is a 24-kDa antimicrobial peptide produced by *Streptococcus bovis* HC5. It is structurally similar to nisin and stable at low pH. It is resistant to some protease like α-chymotrypsin and proteinase K but sensitive to trypsin and pronase E. Bovicin induces potassium efflux through pore formation in the target cell membrane (Mantovani et al. 2002; Paiva et al. 2012). It has been found that when cell is treated with bovicin, there is a significant decrease in cell viabilities of MCF 7 and $HepG_2$ human cell line (Kaur and Kaur 2015).

11.5.10 Smegmatocin

Smegmatocin is a heat labile 75-kD bacteriocin produced by *Mycobacterium smegmatis*. It becomes inactivated at temperature 100 °C or above (Kaur and Kaur 2015). It is sensitive to different protease such as trypsin, chymotrypsin, etc. Smegmatocin showed lethal effect on various cell lines such as human cell lines HeLa S3, mKS-A, Tu-7, AS-II, and HGC 27 from lymph node of gastric cancer showing metastasis. However, HGC27 is less sensitive to smegmatocin AS-II cell line (Van Horssen et al. 2006).

11.5.11 Laterosporulin

It displays human defensin-like structure and is produced by several strains of *Brevibacillus* sp. It is predominantly composed of hydrophobic amino acids. Laterosporulin exhibits cytotoxicity against HT 1080, HeLa H12 99, and MCF-7 cancer cell line in low concentration (10μm) except RWPE-1 action of laterosporulin that involves change in NAD(P)/NAD(P) H ratio and ATP level, which leads to destruction of membrane (Baindara et al. 2017) and finally results in cell death (Baquero et al. 2019).

11.6 Limitations of Bacteriocins as Anticancer Agent

One of the key challenges as therapeutic drug for cancer is the short half-life of these bacterial peptides. Other challenges may involve high cost for large-scale production, less resistance to proteolytic cleavage, deprived delivery system to cancer cells, and lack of well-designed clinical trials (Yaghoubi et al. 2020). Most of the bacteriocins have been studied in vitro and very limited studies under in vivo conditions are available. Bacteriocin, active under in vitro conditions may not show activity in vivo. Thus, validation of these experiments is required under in vivo conditions for application of these bacteriocins as anticancer agent. Therefore, further research is needed in this field to validate the methods and modify the existing natural bacteriocins with the help of genetic engineering to overcome these limitations.

11.7 Future Perspectives

Recent investigations reveal the use of bacterial peptides in cancer therapy due to their unique properties. Bacteriocins displays minimal cytotoxicity to normal cells and more selectivity toward different cancer cell lines, which makes them promising agent for future investigation and clinical trials. Future studies are required to explain the interaction of bacteriocins with different cell surface molecules. A great insight toward in vivo efficacy of bacteriocins for different cell line is also required. Chemical modifications such as amino acid substitution, cyclization, and replacement of labile amino acid may be performed to increase the half-life and stability of bacteriocins (Laliani et al. 2020). The efficacy of bacteriocins can be enhanced by developing hybrid bacteriocins with desired properties. The main aim for the future studies is to substantiate the molecular mechanisms of action of bacteriocins for inventing better and safer therapeutics option for the human use. Such anticancer agents essentially require some rigorous research to invent great therapeutic agent for humankind.

11.8 Conclusion

This chapter has given an account of anticancer potential of few bacteriocins produced by probiotic lactic acid bacteria and other bacteria. It has been observed that bacteriocins have great ability to modulate cancer cell proliferation, differentiation, and apoptosis. These bacteriocins show cytotoxicity against various cancer cell lines and do not affect healthy cells. The structural characteristics of bacteriocins which enhance their anticancer potential and make them promising therapeutics for cancer treatment are: cationic, hydrophobic, oligomerization, low cytotoxicity, and amphipathic structure. There are some unique properties of cancer cell that influence their interaction with bacteriocins, which are, increased expression of negatively charged molecules of membrane surface, overexpression of phosphatidylserine and

o-glycosylated mucin, increased anionic lipid cardiolipin in mitochondrial membrane, and enhanced membrane fluidity. The mechanism of action of bacteriocins mainly includes enhanced apoptosis, cell proliferation inhibition, TNF-α production, membrane depolarization, and angiogenesis inhibition. Thus, selectivity and efficacy of bacteriocins enable them for use of anticancer agents. Few bacteriocins have also shown synergistic effect with other conventional anticancer drugs for their application as chemotherapeutic agents. Altogether, it has been concluded that bacteriocin in general and probiotic bacteriocins in particular have potential applications to develop as anticancer therapeutic agents, provided massive research is conducted in this field.

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Probiotics in Autoimmune and Inflammatory Diseases

12

Vivek P. Chavda, Hitesh Prajapati, Punit Zadafiya, and Moinuddin Soniwala

Abstract

Normal intestinal microflora (gut flora) resides in the gastrointestinal tract, demonstrating mutual symbiotic relationship with host. Intestinal flora contains pathogenic microorganisms, primarily in the large bowel; but most are benign, and some have advantageous effects. Dysbiosis, i.e., the alteration of diversity and composition of the microbiota, contributes to many autoimmune and inflammatory disorders. Studies have demonstrated the great potential of modulating them to treat and prevent diseases. In this chapter the reader will understand the benefits of probiotics in autoimmune, inflammatory, and gastrointestinal disorders like multiple sclerosis, rheumatoid arthritis, type-1 diabetes, ulcerative colitis, gastrointestinal discomfort, improving immune health, relieving constipation, or avoiding the common cold, asthma, alcohol-induced liver injury, etc. Probiotics have provided attractive niche of being administered by different formulations, which may ultimately lead to the widespread use of probiotics in autoimmune and inflammatory disorders, which shall be highlighted here in this chapter with its future prospects.

Keywords

Autoimmune · Inflammatory · Gastrointestinal · Probiotics · Microflora

V. P. Chavda (✉)
Formulation and Development, Dr Reddy's Laboratory (Biotech Division), Hyderabad, Telangana, India

H. Prajapati · P. Zadafiya
Formulation and Development, Biocon Research LTD, Bangalore, Karnataka, India

M. Soniwala
Department of Pharmaceutics, B.K. Modi Government Pharmacy College, Rajkot, Gujarat, India

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12.1 Introduction

Probiotic, a word derived from Latin, means "for life." The health benefits of probiotics are not a new concept; in fact, it is well documented in the older civilizations (McFarland 2015). In the ancient Hindu literature like Rig-Vedas and Puranas, fermented yak milk and its products have been described as one of the panch amruts (Rai et al. 2016). There are many advantages of probiotics that are depicted in Fig. 12.1. Lot of research is directed for the role of probiotics and "gut health". Between 2000 and 2019, large numbers of studies were conducted revolving around probiotics. Only in 2017, a total of around 194 randomized controlled trials (RCTs) were executed, and there were also 49 meta-analyses (Liu et al. 2018). Despite so many added advantages associated with the probiotics, there are certain safety concerns that also need to be taken care like genetic modifications, GI-related as well as some inflammatory conditions. Nowadays there are so many scientists trying to take advantage of probiotics with different formulations and processing technologies (Fig. 12.2). Kaur IP and co-workers have described potential pharmaceutical applications of probiotics (Kaur et al. 2002). Probiotics are the kind of functional food, which have proved their therapeutic potential to prevent diarrhea, improve lactose tolerance, and modulate immunity. They may also have potential to prevent cancer and lower serum cholesterol levels (Kaur et al. 2002).

Changes in gut microbiota and immunological health are mostly associated with persons' age. For gut homeostasis, there is requirement of a mutual symbiotic relationship between gut microbiota and the host immune system (De Oliveira

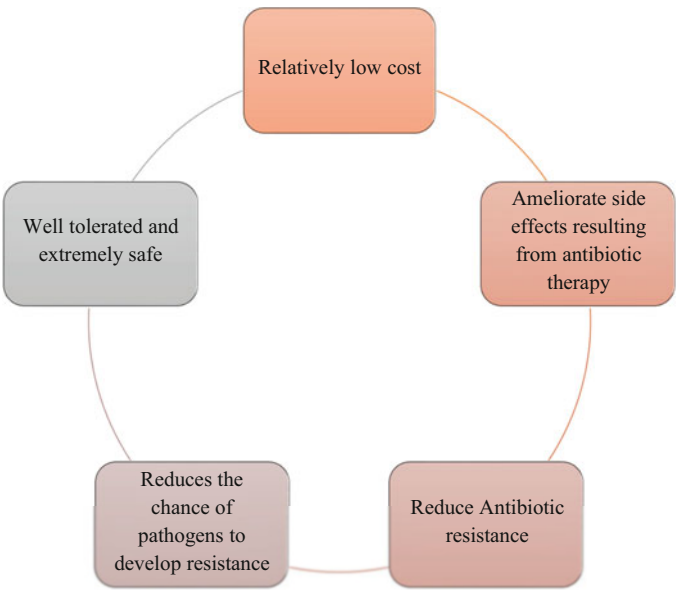

Fig. 12.1 Advantages of probiotic therapy

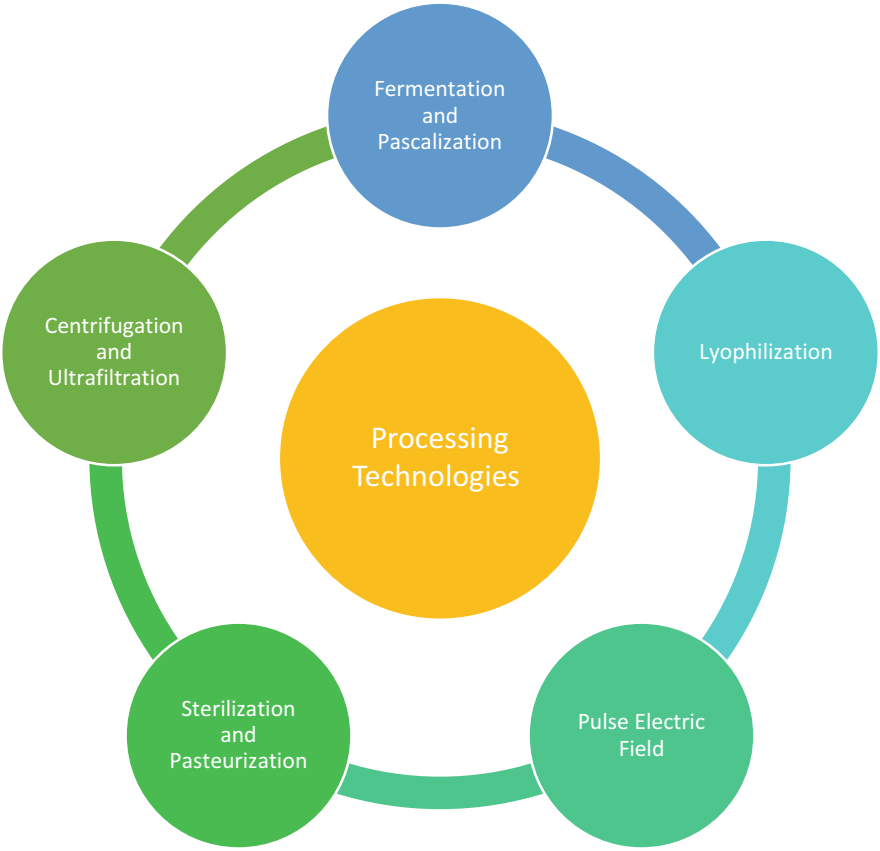

Fig. 12.2 Processing technologies for probiotics

et al. 2017). Here host provides food to microbiota, while microbiota aids in carbohydrate metabolism, vitamin synthesis, and gut-associated lymphoid tissue development, as well as preventing colonization by pathobionts. According to the "hygiene hypothesis," intestinal dysbiosis is the key factor for immune-mediated disorders like eczema, asthma, allergies, and autoimmune diseases (Dargahi et al. 2019). When probiotic supplement is given, it mostly modulates the immune cells and helps in circumventing the pathogenesis of immune disorders (Ford et al. 2014). Imbalance of symbiotic relationship will lead the host to infectious diseases and trigger autoimmune diseases. In this chapter we have tried to unfold the mechanism behind the pathophysiology of such inflammatory and autoimmune conditions. We have tried to encompass different autoimmune and inflammatory disorders and application of probiotic supplement to aid in the treatment, along with a glance at marketed probiotic products for the same.

12.2 Mechanism of Action

The fundamental principle behind the mode of action of probiotics is very complex because of its direct and indirect versatile roles in the cell homeostasis. However, various studies from the past decades had focused on strain-specific effect of probiotics and provided certain important mechanisms of few probiotics. Probiotics can be beneficial to certain pathophysiological conditions in various ways.

12.2.1 By Alternating in the Composition and Activity of the Indigenous Microbiota Temporarily or Permanently

Daily used probiotics include mostly lactic acid bacteria, which have a broad antimicrobial activity such as in one study, where antibacterial activity of *Lactobacillus acidophilus* IBB 801, *Lactobacillus amylovorus* DCE 471, *Lactobacillus casei Shirota*, and *Lactobacillus rhamnosus* GG against *Salmonell*a was solely due to the production of lactic acid (Makras et al. 2006). More specific mechanisms against microbiota include bacteriocin production to inhibit the pathogen growth such as Abp118, and a broad-spectrum bacteriocin produced by Gram-positive bacteria *Lactobacillus salivarius* had enhanced the protection against Listeria, caused by *Listeria monocytogenes* via antagonist mechanism (Corr et al. 2007). Probiotics also induce competition for nutrients between indigenous flora and themselves, for example, administration of probiotic *E. coli* Nissle 1917 assimilated the iron and reduced the colonization of pathogen *Salmonella typhimurium* (Deriu et al. 2013). Probiotics also modulate direct metabolic and systemic metabolic responses, for example, bile salt hydrolase activity by selected probiotics strain has shown high tolerance against bile salts under physiological condition (Begley 2006).

12.2.2 By Enhancing the Function of Epithelial Barrier

In this category, probiotics action include tightening of epithelial junction, which in turn leads to reduction in the permeability of barrier, for example, administration of *Lactobacillus plantarum* in healthy individual induced the translocation of zonula occludens (ZO)-1 to tight junction (TJ) region, which resulted into formation of paracellular seals between the epithelial cells and reduction in permeability of epotheilium barrier (Karczewski et al. 2010). Another study, where a probiotic *Lactobacillus rhamnosus* GG-derived soluble protein p40 upregulates the catalytic activity of a disintegrin and metalloproteinase domain containing protein 17 (ADAM17), which resulted into release of heparin-binding (HB) epidermal growth factor (EGF) followed by transactivation of EGF receptors and prevention of apoptosis of intestinal epithelial cells (Yan et al. 2013).

12.2.3 By Regulation of the Immune System

Probiotics have the tendency to interact with pattern recognition receptors (PRRs) of the immune system such as toll-like receptors (TLRs), which have effects on various immune cells of the immune system such as macrophages, monocytes, and dendritic cells, which in turn further modify the balance of T-helper and T-regulatory cells or antibody production by B-cells. Various studies reported that interplay between PRRs of gastrointestinal mucosa and bacterial cell surface macromolecules such as polysaccharides, lipoteichoic acids, and surface appendages including flagella, pili, and fimbriae resulted into mitogen-activated protein kinases (MAPK) or phosphoinositide 3-kinase (PI3 kinase) or protein kinase C (PKC) or NF-Kβ signaling, which regulates the proliferation of T-helper and T-regulatory cells or other immune cells and transforms pathogenicity into mutualism (Lebeer et al. 2010).

12.2.4 By Signaling via the Central Nervous System

Several direct and indirect mechanisms of probiotic signaling through the central nervous system include tryptophan-derived products, γ-amino-butyric acid (GABA), which help in the reduction of brain-related disorders. Administration of *Lactobacillus rhamnosus* to healthy male BALB/c mice has induced changes in GABA-A and GABA-B receptor subtypes in specific brain regions, which resulted in reductions in anxiety- and depression-related behaviors (Janik et al. 2016).

12.3 Inflammatory Diseases

Inflammatory diseases are conditions, which are characterized by common inflammatory pathways leading to inflammation, resulting from dysregulation of the normal immune response. Inflammation is a critical response to potential danger signals and damage in organs of our body, but under certain circumstances, inflammation can take two basic forms, acute or chronic. Hence, inflammation is the primary driver of many medical disorders and autoimmune diseases, including ankylosing spondylitis, psoriasis, psoriatic arthritis, rheumatoid arthritis, Behcet's disease, arthritis, and inflammatory bowel disease (IBD). All inflammatory diseases can cause end organ damage and are associated with negative effects on the structure and function of cell organs or body parts and/or death.

Following are certain examples of inflammation diseases:

12.3.1 Ankylosing Spondylitis

Ankylosing spondylitis (AS) is a consequence of a long-term inflammation of the joints of the spine. Males are more often affected than females. Typically, AS affects the joints that connect the spine and pelvis (Edavalath 2010). AS also affects other

joints such as the shoulders or hips (Edavalath 2010). The signs and symptoms of AS involves eye and bowel problems, back pain, stiffness of the affected joints, chronic dull pain in the lower back, weight loss, fever, or fatigue, loss of spinal mobility and chest expansion, and extension of the lumbar spine (McVeigh et al. 2006). The mechanism involves implication of tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1). There is an association of AS with human leukocyte antigen (HLA) that involves interaction of CD8 T cells with HLA-B (Longo 2012). This interaction involves no self-antigen but occurs due to the antigens from intracellular microorganisms (Braun et al. 1998). Infections caused by the bacteria such as *Salmonella*, *Shigella*, *Yersinia*, and *Campylobacter* are generally associated with pathogenesis of AS (Madsen et al. 2001). Probiotics having *Bacteroides fragilis* have the ability to induce anti-inflammatory responses by activating the T-regulatory cells against its polysaccharides A molecule. T-regulatory cells in turn secrete the IL-10, which subsequently inhibits the Th17 cell response (Round and Mazmanian 2010). One of the commercially available VSL#3 probiotics with several *Lactobacillus* and *Bifidobacterium* species has ability to reduce TNF-α and interferon-gamma (IFNγ) level, which are associated with the ankylosing spondylitis pathogenesis (Asquith et al. 2014).

12.3.2 Psoriatic Arthritis

Psoriatic arthritis is a long-term inflammatory arthritis that occurs in people affected by the disease psoriasis (Freedberg and Fitzpatrick 2003). The joints of the hand that are involved in psoriatic arthritis are the proximal interphalangeal (PIP), the distal interphalangeal (DIP), the metacarpophalangeal (MCP), and the wrist (James et al. 2005). The characteristics of psoriatic arthritis are also involved in the swelling of fingers and toes with a sausage-like appearance in association with small depression in the nail, thickening of nail, and detachment of nail from the nailbed (Ritchlin et al. 2017). Skin becomes red, scaly, and itchy because of psoriasis before the onset of psoriatic arthritis. The signs and symptoms of psoriatic arthritis involve pain, swelling, stiffness in joints, and redness in the joints (Amherd-Hoekstra et al. 2010). The mode of mechanism of psoriatic arthritis is unknown, but the involvement of human leukocyte antigen (HLA)-B27 indicates the genetic association with disease progression (Rahman and Elder 2005). *Bacteroides fragilis* has the ability to induce anti-inflammatory responses by activating the T-regulatory cells against its polysaccharides A molecule. T-regulatory cells in turn secrete the IL-10, which subsequently inhibits the Th17 cell response (Round and Mazmanian 2010).

12.3.3 Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an autoimmune or inflammatory disorder that mostly affects body joints, which results in inflammation and thickening of the joint capsule (Handout on Health: Rheumatoid Arthritis 2014). It also affects the bone and

cartilage. The signs and symptoms of RA involve swollen and painful joints, stiffness, osteoporosis, feeling tired, fever, depression, mental difficulties, and trouble in working (Cutolo et al. 2014). The mechanism of RA involves the generation of autoreactive cytotoxic T-lymphocytes (CTLs) against the rheumatoid factors, which in turn leads to filtration or accumulation of CTLs at various body joints, followed by type-IV hypersensitivity. The formation of rheumatoid factors and IgM–IgG complexes activates the Fc receptors in the cells of joint tissue, which results into type-III hypersensitivity reaction (Holmes 1999). Probiotic bacteria like *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Bacillus coagulans*, *Lactobacillus reuteri*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum* have shown the ability to treat RA (Pineda et al. 2011).

12.3.4 Behcet's Disease

Behçet's disease (BD) is a type of inflammatory disorder, which affects multiple parts of the body (Zeidan et al. 2016). The signs and symptoms of BD involve mouth ulcer, genital ulcers, inflammation in the eyes, in the brain, or spinal cord, blood clots, blindness, and arthritis (Ferizi et al. 2018). The symptoms will mostly come and go. The mode of mechanism of BD is an autoimmune followed by inflammation in which overactive immune system targets the patient's own body. The involvement of a subset of T cells (Th17) seems to be important (Hatemi et al. 2012). Heat shock proteins (HSPs) from some bacteria serve as a "danger signal" to the immune system in the BD. These HSPs from bacteria are similar to humans (Direskeneli 2013). The anti-HSP60 and anti-HSP65 antibodies produced against HSPs from *Streptococcus sanguinis*, *Streptococcus pyogenes*, and *Mycobacterium tuberculosis* can also attack human HSPs, which in turn results into abnormal immune response (Tanaka et al. 1999). Bacterial family such as Actinobacteria, Lactobacillaceae, and Coriobacteriaceae had showed larger positive effects in patients with BD (Shimizu et al. 2016).

12.3.5 Arthritis

Arthritis is a disease that affects joints with chronic inflammation. There are more than 100 types of arthritis (Athanasiou et al. 2013). The signs and symptoms of arthritis involve swelling of joints, joint pain stiffness, redness, inability to move affected joints, malaise, fatigue, poor sleep, and tenderness. The most common forms are osteoarthritis and rheumatoid arthritis. Osteoarthritis affects the fingers, knees, and hip region of the body, whereas rheumatoid arthritis affects the hands and feet. Other forms of arthritis are gout, lupus, and septic arthritis (NIAMS 2014). Probiotic bacteria like *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus reuteri*, and *Lactobacillus acidophilus* have the ability to treat arthritis (Pineda et al. 2011).

12.3.6 Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is a disorder that involves chronic inflammation of digestive tract (Baumgart and Carding 2007). There are two types of IBD:

(a) Ulcerative colitis: The classic features of ulcerative colitis involve long-lasting inflammation and ulcers in the innermost lining of large intestine or colon and rectum.
(b) Crohn's disease: The classic feature of Crohn's disease involves inflammation of the lining of digestive tract, which in turn spreads deep into affected tissues.

The symptoms of IBD vary depending on the severity of inflammation and target location where it occurs (Wang et al. 2012). The signs and symptoms of both ulcerative colitis and Crohn's disease involve severe diarrhea, abdominal pain and cramping, fatigue and weight loss, fever, blood in the stool, and reduced appetite. The mode of mechanism of IBD is unknown. One possible cause is an immune system malfunction (Stein et al. 2010). When immune system activates against invading virus or bacterium for protection, an abnormal immune response causes the immune system to attack the cells in the digestive tract also, which leads to IBD. Probiotics bacteria including *Lactobacillus rhamnosus*, *Bifidobacteria*, and *Saccharomyces boulardii* have ability to treat the IBD by increasing secretory immunoglobulin A secretion, decreasing proinflammatory cytokines, and inducing the upregulation of regulatory cytokines and T cell apoptosis (Katz 2006).

12.4 Autoimmune Disorder

An autoimmune disease is an abnormal immune response to a normal body part. There are various types of autoimmune diseases including Grave's disease, systemic lupus erythematosus (SLE), multiple sclerosis, rheumatoid arthritis, diabetes mellitus type 1, psoriasis, and celiac disease (McFarland 2015; Rai et al. 2016). As per a generalization, women are more commonly affected than men. Autoimmune diseases have various pathological effects that include damaging or destruction of tissues, altered organ growth, or altered organ function (Liu et al. 2018). Immune system generally produces both T cells and B cells that have the capability to react with self-molecules of body, but these self-reactive cells are usually passed to clonal Energy state in which they are silently removed from their role within the immune system due to overactivation or removed from their role within the immune system by regulatory cells. When any one of these mechanisms fails, there is a possibility that self-reactive cells become functional within the immune system, which in turn leads to autoimmune disorders. Autoimmune disease can be either random or systematic in which autoreactive antibodies or autoreactive cytotoxic T cells (CTLs) attack self-antigen of normal organs or tissues, which results into abnormal immune response and autoimmune disorders. Following are certain examples of autoimmune diseases:

12.4.1 Grave's Disease (GD)

Grave's disease is an autoimmune disease that affects the thyroid organ, causing hyperthyroidism. Grave's disease has signs and symptoms such as swollen thyroid, hair loss, increased appetite, hyperactivity, insomnia, muscle weakness or paralysis, sleeping problem, diarrhea, weight loss, irritability, itching, heat intolerance, and fast heartbeat. Autoreactive antibodies, called thyroid-stimulating immunoglobulins (TSIs), are produced in Grave's disease, which have a similar effect to thyroid-stimulating hormone (TSH). These TSI antibodies recognize and bind to thyroid-stimulating receptor (TSR), which causes the thyroid gland to produce excess thyroid hormones such as thyroxine (T4) and triiodothyronine (T3) by acting as agonist to TSR (Brent 2008). Increase in anti-*Saccharomyces cerevisiae* antibodies (ASCA), production of antibodies against *Yersinia enterocolitica* and *Helicobacter pylori*, and significant decrease in bacteroides can be found in patient with Grave's disease. It is required to understand the role of microbiota and GD in much deeper level, so that manipulation of microbiota, i.e., with probiotics can be used in treatment of GD (Opazo et al. 2018). Probiotics supplementation was found to prevent serum hormonal fluctuations (levothyroxine (LT4)) by a mixture of highly charged *Lactobacilli* and *Bifidobacteria* (Spaggiari et al. 2017).

12.4.2 Systemic Lupus Erythematosus (SLE)

Systemic lupus erythematosus (SLE), also known as lupus, is an autoimmune disease in which the immune system attacks healthy tissue in many parts of the body. The signs and symptoms of SLE include painful and swollen joints, fever, chest pain, muscle pain, fatigue, hair loss, mouth ulcers, swollen lymph nodes, feeling tired, red rash, and butterfly mark on the face. The mechanism of SLE involves an immune response by autoreactive antibodies (most commonly antinuclear antibodies) against antigen of own healthy tissues. The healthy tissues affected due to autoreactive antibodies include skin, blood, muscle, bones, heart, lungs, kidney, eyes, etc. The autoreactive antibodies bind to proteins of cell nucleus and form the immune complexes, which in turn cause a type-III hypersensitivity reaction (NIAMS 2015). Dysbiosis of microbiota is also involved in pathogenesis of SLE. Majorly, bacteria phylum *Firmicutes* reduction and increase of *Bacteroides* were detected. Reduction in the level of bacterial family *Lactobacillaceae* and increase in *Lachnospiraceae* and *Clostridiaceae* were found. *Lactobacillus paracasei* GMNL-32, *Lactobacillus reuteri* GMNL-89, and *Lactobacillus reuteri* GMNL-263 have shown promising results in T regulation (Treg) cells in animal model, and mixture of these strains may be used as adjuvant treatment for SLE patients (De Oliveira Gislane 2012). Administration of probiotic bacteria *Bifidobacteria* may reduce inflammatory response and production of antireactive antibodies (Al-Salami et al. 2012). Studies have shown that administration of *B. bifidum* LMG1395 could reduce overactivation of $CD4^+$ lymphocytes, whereas Clostridia *Ruminococcus obeum* DSM25238 and *Blautia coccoides* DSM935 help to maintain Th1 balance (Esmaeili

et al. 2017). *L. rhamnosus* GG and *L. delbrueckii* subsp. *lactis* have shown promising effect in reducing SLE severity in animal model by enhancing effect of Treg and reduction in inflammatory cytokines (Khorasani et al. 2018).

12.4.3 Multiple Sclerosis

Multiple sclerosis (MS) is an autoimmune disease in which the insulating covers of nerve cells in the brain and spinal cord are damaged, which results into disruption of the nervous system to communicate (NINDS 2015). This disease includes a range of signs and symptoms including physical, mental, and sometimes psychiatric problems (Compston and Coles 2008). Specific symptoms can include double vision, blindness in one eye, muscle weakness, trouble with sensation, or coordination. The mechanism of MS involves the generation of autoreactive CTLs against the myelin sheath of nerve tissue, which enter into the brain by disrupting blood–brain barrier and damage the myelin sheath of nerve tissue and cause the demyelination, which results into the paralysis or loss of muscle contraction. Studies show that MS patient has imbalance of gut microflora. Especially, reduced level of *Bacteroides*, *Parabacteroides*, *Prevotella*, and *Lactobacillus* genera and higher level of *Akkermansia* (*A. calcoaceticus and A. muciniphila*), *Blautia*, *Ruminococcus*, and *Bifidobacterium*. Oral administration of *B. fragilis* polysaccharides has shown to be helpful in MS by enhancing IL-10 secretion (Opazo et al. 2018). Orally administered eggs of nonpathogenic helminth *Trichuris suis ova* (TSO) have shown favorable results in MS by increasing IL-4 and IL-10 cytokines. Studies report reduction in inflammatory markers in MS patient by probiotic supplement containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus fermentum*, and *Bifidobacterium bifidum* (Opazo et al. 2018). Probiotic supplements containing *L. acidophilus*, *L. casei*, *L. fermentum*, and *B. bifidum* reduce the expression of m-RNA of inflammatory markers of IL-8 and TNF α in MS patient. It is reported that yeast *Saccharomyces boulardii* (SB) is more effective than *Lactobacillus*- and *Bifidobacterium*-based probotics (Aghamohammadi et al. 2019).

12.4.4 Diabetes Mellitus (Type I Diabetes)

Type I diabetes, also known as juvenile diabetes, is an autoimmune disorder in which insulin is not sufficiently produced by the pancreas (WHO 2016). The classic signs and symptoms involve frequent urination, increased thirst, increased hunger, weight loss, blurry vision, poor wound healing, and tiredness. The mechanism of type I diabetes involves the destruction of the insulin-producing beta cells in the pancreas by autoreactive antibodies. The destruction of beta cells results into low level of insulin and hyperglycemic condition, which in turn lead to type-I diabetes (NIDDK 2014). Gestational diabetes mellitus is nowadays becoming very common due to changes in life style. It has deleterious impact during pregnancy and in later stage of life (Barrett et al. 2012). A scientific group have evaluated the usage of

probiotic supplements in diabetic patients and concluded that it may be considered as an adjunct treatment for glycemic control in these patients (Kijmanawat et al. 2019). Probiotic supplements have been shown to improve metabolism by increasing host insulin sensitivity and cholesterol metabolism and also have a beneficial effect on the immune system (Dolatkhah et al. 2015). Use probiotic supplements for the prevention of gestational diabetes (Barrett et al. 2012), wherein significant improvement in HbA1c and fasting insulin in type 2 diabetes patients has been observed (Yao et al. 2017).

12.4.5 Psoriasis

Worldwide ~2% of people are affected with psoriasis. In psoriasis, new skin cells are formed too quickly, generally 10 to 12 times faster than normal. Psoriasis is a disease characterized by patches of abnormal skin, which occurs due to combined effect of acute inflammation and abnormal immune response. The skin patches are typically red, dry, itchy, and scaly (Menter et al. 2008). Malfunction of innate and adaptive immune system, genetical factors, and modification of skin microbiota lead to uncontrolled skin cell proliferation and differentiation, which in turn develop psoriasis (Rendon and Schäkel 2019). *Lactobacillus salivarius* LA307 and *Lactobacillus rhamnosus* LA305 were found reduced and may even prevent chronic skin inflammation and reduce biomarkers of inflammation (Eske 2019).

Purple color patches may be present in people with darker skin (Priscilla et al. 2015). There are five main types of psoriasis: plaque, guttate, inverse, pustular, and erythrodermic. Plaque psoriasis, also known as psoriasis vulgaris, is a predominant form among all types of psoriasis with red patches and white scales. Plaque psoriasis affects the back of the forearms, shins, navel area, and scalp (Boehncke and Schön 2015). Guttate psoriasis involves drop-shaped lesions. Pustular psoriasis occurs as small pus-filled blisters (Jain 2012). Inverse psoriasis involves the formation of red patches in skin folds. Erythrodermic psoriasis occurs with spreading of the rashes and can develop from any of the other types of psoriasis (Palfreeman et al. 2013). The signs and symptoms of psoriasis involve pits in the nails or changes in nail color, plaques on the elbows, knees, scalp, and back (Colledge et al. 2010), inflammation and exfoliation of the skin over most of the body surface, dryness, itching, swelling, and pain. The psoriasis disrupts the body's ability to regulate temperature and perform barrier functions (Stanway 2014). Psoriasis is characterized by an abnormally excessive and rapid growth of the epidermal layer of the skin with abnormal production of skin cells (especially during wound repair) and an overabundance of skin cells (Raychaudhuri et al. 2014). The mode of mechanism involves premature maturation of keratinocytes induced by an inflammatory cascade in the dermis involving dendritic cells, macrophages, and T cells (three subtypes of white blood cells) (Cedeno-Laurent et al. 2011). These immune cells migrate from the dermis to the epidermis and secrete inflammatory cytokines such as IL-36γ, TNF-α, IL-1β, IL-6, and IL-22 (Baliwag et al. 2015). One hypothesis is that psoriasis occurs due to defect in regulatory T cells and in the regulatory cytokine IL-10 (Nestle et al.

2009). IL-22 acts in combination with IL-17 to induce keratinocytes to secrete neutrophil-attracting cytokines, which in turn induce psoriatic lesion. Streptococci species were associated with chronic plaque psoriasis due to production of M-protein, which may have the ability to mimic keratin determinants, followed by psoriatic T cell activation (McFadden et al. 1991). Apart from that skin microbiota such as *Corynebacterium*, *Propionibacterium*, *Staphylococcus*, and *Streptococcus* may have role in the pathogenesis of plaque psoriasis. *Lactobacillus paracasei* and *Lactobacillus pentosus* have the ability to reduce psoriasis-related pro-inflammatory cytokines such as TNF-α and several ILs (Gueniche et al. 2013).

12.4.6 Celiac Disease

Celiac disease is an immune disease, which damages small intestine. Generally, 0.5–1% of global population, predominantly women, are suffering from celiac disease. Symptoms vary from person to person and involve dysfunction of digestive system, diarrhea, abdominal pain, irritation, and depression (Tye-Din et al. 2018). Pathogenesis of CD involves predisposition of genetic element (human leukocytes antigen [HLA]-DQ2 and HLA-DQ8), overactivity of adaptive immune system (CD4 + T cells) and innate immune system (IL-15 and interferon α), role of environmental trigger (gluten), role of autoantigen (tissue transglutaminase [tTG]), and imbalance of microbiota in gut (Caio et al. 2019). It has been proved that there is a decrease in *Bifidobacterium* spp. and increase in the number of *Bacteroides* spp., which can be circumvented by proper balanced probiotic supplements (Cristofori et al. 2018). Future research efforts are required to determine the relationships between CD and microbiota (both oral and intestinal) to improve the composition of GFD for restoring the gut dysbiosis as a preventative or therapeutic approach for CD (De Angelis et al. 2016).

12.4.7 Allergies

Allergy, also termed type I hypersensitivity, is defined as a "disease following a response by the immune system to an otherwise innocuous antigen" (Prakash et al. 2014). It is mainly associated with the gut immune system and for the same no proper treatment is available yet. It is associated with some environmental antigen and is linked to the innate immunity of the host (Gourbeyre et al. 2011). Alteration in innate immunity is mainly driven by the improvement in the mechanism of pathogen destruction by modulation of the immune system. By stimulating autochthonous bacteria metabolism, probiotics improve the immune system function (Prakash et al. 2014). *Lactobacilli* and *Bifidobacteria* as a functional food have proved health benefits to the host (Prakash et al. 2014). In recent studies, probiotic formulations demonstrated the capability to successfully modulate allergic rhinitis, IgE-sensitized (atopic) eczema, asthma, and food-related allergies (Ozdemir 2010). A number of probiotic mechanisms of action are involved in controlling hypersensitivity

responses, many of which are still not yet understood. Formulation scientists have found attractive niche for microencapsulation of probiotic to treat and prevent allergies (Prakash et al. 2014). For the clinical success of probiotic therapy, various factors such as type of bacterium and their combination, dosing regimen, delivery method, and other underlying host factors, e.g., the age and diet of the host need to be considered while grafting the formulation (Ozdemir 2010).

12.5 Marketed Probiotics

As per one estimation, the probiotics market is USD 49.4 billion in 2018, which is projected to be around USD 69.3 billion by 2023 (Marketsandmarkets 2019). The 2012 National Health Interview Survey (NHIS) showed that about 4 million (1.6 percent) US adults had used probiotics or prebiotics in the past 30 days. In terms of dietary supplement other than vitamins and minerals, probiotics ran the third in the US. The 2012 NHIS also showed that 300,000 children aged 4 to 17 (0.5 percent) had used probiotics or prebiotics in the 30 days before the survey (NCCIH 2019). There are so many probiotic products available in the market, but here we have highlighted only those marketed probiotics catered for autoimmune and inflammatory disorders (Table 12.1).

12.6 Hurdles and Road Ahead: The Future of Probiotics

Traditional probiotic strains have a long history of safe and effective use in a range of diseases, and with each passing day they are finding new therapeutic applications, but the fact that a complete absence of risk does not exist with the use of microbial systems cannot be overlooked (Saarela 2000). When we talk about autoimmune disease, its pathophysiology is almost always amalgamated with dysfunction in immune system, and 80 percent of our immune tissue is in the digestive tract. Once you have inflammation, you are at risk of developing a condition known as "metabolic endotoxemia" (Shomon 2019). Rise in this kind of toxins may increase the triglyceride levels and increase levels of cytokines and inflammation, which in turns leads to immune dysfunction and inflammatory conditions. When such condition exists in the body, it will increase the intestinal permeability for the lager toxin, which can be controlled through probiotic supplement. It is now well known that the gut microbiota and their metabolites play a key role in the pathogenesis of inflammatory and autoimmune diseases. Several randomized controlled trials have now shown that microbial modification by probiotics may improve gastrointestinal symptoms and multiorgan inflammation in rheumatoid arthritis, ulcerative colitis, and multiple sclerosis (Liu et al. 2018). Here in this chapter we discussed all aspects of autoimmune and inflammatory disorders with the proposed mechanism by which probiotic supplements improve the function. Still lot more work needs to be imparted toward the safety concern associated with the probiotics' usage. A platform approach needs to be devised for the careful selection of bacterial strain and its combination.

Table 12.1 Marketed probiotics for autoimmune and inflammatory disorders

Sr. no.	Product name	Probiotic bacteria present/ main ingredients	Application/therapeutic use	Manufacturer/ source	Dosage form/remark/ comment	Reference
1	ULTRA-15 PROBIOTICS	*L. acidophilus*, *B. bifidum*, *B. breve*, *B. infantis*, *B. lactis*, *B. longum*, *L. casei* subsp. *casei*, *L. fermentum*, *L. gasseri*, *L. plantarum*, *L. reuteri*, *L. rhamnosus*, *L. salivarius*, *S. thermophilus*, *B. coagulans*, *L. paracasei*, *L. bulgaricus*, *L. helveticus*, prebiotic FOS	Restore digestive and immune health	Island's Miracle (USA)	Dietary supplements, delayed release capsule (DR CapTM), for women and men	Vitamiracle (2019)
2	ULTRA-70 PROBIOTICS	Probiotic blend: *L. acidophilus*, *L. casei*, *L. salivarius*, *L. plantarum*, *L. bulgaricus*, *L. rhamnosus*, *L. paracasei*, *L. brevis*, *L. gasseri*, *L. lactis*, *B. infantis*, *B. lactis*, *B. breve*, *B. bifidum*, and *B. longum*	Boost immune system and improve digestive health	Vita Miracle® (USA)	Dietary supplements, delayed release capsule (DR CapsTM), for women and men	Vitamiracle (2019)
3	Ultra-30 women's probiotics	Intestinal health blend: L-glutamine, inulin (FOS), apple pectin	Boost the immune system, promote digestive, urinary and vaginal health, can reduce occurrence of gas, bloating, diarrhea, constipation, and bowel irritations associated with digestive disorders, strengthen immunity, and	Vita Miracle® (USA)	Dietary supplements, delayed release capsule (DR CapsTM), especially for women	Vitamiracle (2019)

			reduce inflammation, ulcers and allergies			
4	ULTRA-50 PROBIOTICS	Probiotic blend: *L. acidophilus*, *L. rhamnosus*, *L. fermentum*, *B. bifidum*, *B. longum*, *B. infantis*, *L. gasseri*, *B. coagulans*, *B. lactis*, *L. reuteri*, *L. casei*, *L. paracasei*, *L. bulgaricus*, *L. plantarum*, *L. salivarius*, *S. thermophilus*, *B. breve*, and *L. helveticus*	Improve overall health and energy level and improve digestive system	Vita Miracle® (USA)	Dietary supplements, delayed release capsule (DR CapsTM), especially for women, men and kids	Vitamiracle (2019)
5	ULTRA-30 PROBIOTICS	Urinary and vaginal health herbal blend: organic cranberry extract, goldenrod, bearberry leaf (Uva Ursi)	Maximize digestive and immune health, reduce gas, bloating, cramping, and other IBS symptoms, decrease inflammation, and decrease allergy	Vita Miracle® (USA)	Dietary supplements, delayed release capsule (DR CapsTM) for women and men	Vitamiracle (2019)
6	Ultimate FloraTM Probiotic	Proprietary enzyme blend: protease, cellulase, hemicellulase, amylase, glucoamylase, lactase, invertage, and serrapeptase	Supports digestive and immune health and supports healthy vaginal microflora	Renew life formulas Inc. (USA)	Capsule for women	Renewlife (2019)
7	Bio-K Plus®	Prebiotic: fructooligosaccharides (FOS)	Support healthy digestive system and sustain and boost your immune system	Bio-K Plus International Inc. (Canada)	Enteric coated capsule	Biokplus (2019)
8	Align® PROBIOTIC	Proprietary probiotic blend: *L. acidophilus*, *B. bifidum*, *B. breve*, *B. infantis*, *B. longum*, *B. lactis*, *L. casei*	Naturally helps in occasional abdominal discomfort, gas, and bloating and helps	P&G (USA)	Capsule	The Healthy (2019)

(continued)

Table 12.1 (continued)

Sr. no.	Product name	Probiotic bacteria present/ main ingredients	Application/therapeutic use	Manufacturer/ source	Dosage form/remark/ comment	Reference
		subsp. *casei*, *L. fermentum*, *L. gasseri*, *L. plantarum*, *L. reuteri*, *L. rhamnosus*, *L. salivarius*, *S. thermophilus*, *B. coagulans*, *L. paracasei*, *L. bulgaricus*, *and L. helveticus*	promote and support a healthy digestive system.			
9	Saccharomyces boulardii + MOS	Prebiotic: FOS	Helps protect and maintain normal intestinal microflora during travel or intake of certain medications. MOS can discourage bacteria from adhering to the epithelial cells and reduce their proliferation	Jarrow Formulas® (USA)	Delayed release capsule (DR CapsTM)	Jarrow (2019)
10	Culturelle® PROBIOTICS Kids	Proprietary probiotic blend: *L. acidophilus*, *B. bifidum*, *B. breve*, *B. infantis*, *B. longum*, *B. lactis*, *L. casei* subsp. *casei*, *L. fermentum*, *L. gasseri*, *L. plantarum*, *L. reuteri*, *L. rhamnosus*, *L. salivarius*, *S. thermophilus*, *B. coagulans*, *L. paracasei*, *L. bulgaricus*, and *L. helveticus*	Support digestion and healthy immune system for kids	i-Health, Inc. (USA)	Powder	Culturelle (2019)

11	BlueBIotics Ultimate Care	Prebiotic: FOS	Improved digestion (gut health, immune support, improved mood, constipation/ diarrhea, increased energy, reduction of blood pressure, reduced anxiety, improved IBS symptoms, reduced inflammation of the gut, and improved skin health	Blue Biology (USA)	Dietary supplement, vegetarian capsule	Bluebiology (2019)
12	Probiotic100B	Culture blend: *L. acidophilus*, *L. rhamnosus*, *L. plantarum*, *L. gasseri*, *L. casei*, *L. salivarius*, *L. paracasei*, *L. brevis*, *B. lactis*, and *B. longum*	Digestive support	Ayush Herbs© Inc. (USA)	Capsules for both men and women	Ayush (2019)

Attempts need to be drawn for the formulation of probiotics with different dosage form to prolong it action as well as protection in the gut without compromising its efficacy. As the boon in the biotechnology field, one can also link genetic engineering to develop new safe strain as well as combination of products to get more benefit out of it.

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Role of Probiotics in Rheumatoid Arthritis

13

Rahul Shukla, Munindra Ruwali, N. Sharath Pawar, and S. J. S. Flora

Abstract

Lifestyle changes have affected the health of many individuals, majority being in industrialized nations compared to developing nations. Epithelial and mucosal permeability potentially mediates and influences immune tolerance to residing local microbiome. Lifestyle changes and dietary habits affect the local microbiome and finally lead to immunological imbalances. Diet which has great impact on microbiome of human intestines is associated with inflammation at local site and enhances the permeability of pro-inflammatory lymphocytes and cytokines into systemic circulation and leads to spread of various inflammatory mediators to distant joints. Recently, clinical trials which reported an administration of *Lactobacillus casei* 01 to rheumatoid arthritis patients have significantly reduced level of inflammatory cytokines and alleviation of symptoms. This chapter will emphasize on literature related to relationship of intestinal microbiome with arthritis progression and role of probiotics in management of rheumatoid arthritis.

Keywords

Probiotics · Arthritis · Symbiotic · Gut · Microbiome · Lactobacillus

R. Shukla (✉) · N. Sharath Pawar
Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research-Raebareli, Lucknow, Uttar Pradesh, India

M. Ruwali
Amity Institute of Biotechnology, Amity University Haryana, Gurgaon, Haryana, India

S. J. S. Flora
Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research-Raebareli, Lucknow, Uttar Pradesh, India

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13.1 Introduction

Millions of commensal as well as symbiotic microorganisms are residing in the human body (Dhanoa 2019) and contain bacteria equivalent to human cells (Sender et al. 2016). Colonized locations constitute the skin, upper respiratory tract, oral cavity, intestinal tract and genital tract (Dhanoa 2019). Right from the birth the colonization process begins in the baby on its first exposure to vaginal canal of mothers (Jethwa and Abraham 2017; Dhanoa 2019). Various factors affect the different composition of gut microbiome such as infection, drug use, age, nutrition, and stress (Jethwa and Abraham 2017).

Human microbiome highly governs the expansion and protection of immune system (Bedaiwi and Inman 2014). The gut generally provides safety against antigens from various microorganisms (Dhanoa 2019). Presence of healthy microbiota is helpful in prevention of diseases. Various methods are employed for protecting the gut microbiome and pathogens by host immune system with the help of mucus layer and tight junctions (Jethwa and Abraham 2017). Arousal of the early innate reaction in rheumatoid arthritis (RA) requires dendritic cells, macrophages, cytokines, natural killer cells as well as γ/δ T cells (Arend 2001; Handa et al. 2019). The dendritic cells along with macrophages are responsible for stimulating innate immune system for obtaining quick effector response. RA is highly antigen-specific and is affected by adaptive immune response which is supported by activation of T cells. Articular destruction in RA patients is due to contribution of well-known pro-inflammatory cytokines which are IL-1, GM-CSF, TNFα, IL-15, IL-18 and IL-12 (Brennan and Mcinnes 2008; Shukla et al. 2019). One must be mainly emphasized on diet therapy especially for RA for curing the microbiome and rebuilding of a healthy immune system.

Even though most of the rheumatic diseases are composed of substantial heritable moieties, influencing genetic factors may require certain level of environmental triggering to start the immune-pathological events which are responsible for disease manifestation (Bedaiwi and Inman 2014). Researchers had reported various examinations referring the linkage between gut microbiome and rheumatic diseases (Yeoh et al. 2013). Many organisms show defending role of gut microbiome against several autoimmune disorders, as illustrated by nondiabetic mice with an overexpression of bacteroidetes (Maslowski and MacKay 2011). The data from various reports tried to fill gap and act as link for correlating rheumatic disorder progression with gut microbiomes. Presence of endogenous gut microbes affects the host neuroendocrine homeostasis and metabolism which controls the hosts body weight (Sanz et al. 2010). In this manuscript, we tried to gather the information on the probiotics role in confederation of rheumatoid arthritis and detailed study of its mechanisms, pathways, clinical and animal studies. Authors had reviewed many literatures available on various scientific search engines to find the suitable mechanism with an exposure to find the probiotics role in management of rheumatoid arthritis. Till date *Lactobacillus* and its strains are extensively studied for therapeutic potentials in rheumatoid arthritis.

13.2 Probiotics

Greek meaning for term "probiotic" is "for life" although it had different meanings with advancement in life sciences. Greeks and Romans were the first to utilize fermented foods and that is why origin of probiotics is from them. Ancient texts, like Old Testament, reported the use of first living microorganisms in foods as fermented milks (Anadón et al. 2016). Fermented milk intake is till date continued in different forms. Probiosis is defined as intake of fermented dairy foods having positive impact by incorporating cultures of lactic acid bacteria (LAB) and balancing intestinal microflora. Parker coined the term probiotics in 1974. According to Parker, probiotics are defined "as organisms and substances which contribute for balancing intestinal microbiome". This highlights an important role of gut microflora in protecting host from various diseases (Fuller 1992).

According to Food and Agriculture Organization (FAO), probiotics are defined as live microorganisms which when administered in a passable amount to host, provides health benefits (Fao et al. 2002). Metchnikoff assumed that microorganisms which are lethal and destructive in nature residing in body can be changed to useful microbes through diet (Dhanoa 2019). General classification of the bacteria residing in the human is beneficial, harmful, or that exhibits an intermediate property. *Clostridium, Enterobacteriaceae, Veillonella* and *Proteus* are destructive bacteria, whereas beneficial bacteria include *Bifidobacterium* and the *Lactobacillus* genera which are Gram-positive and non-spore forming rods (Gill and Prasad 2008). The regulation of the immune system by consumption of beneficial bacteria is primarily helpful to health. Probiotics are reported to have an immunomodulatory effect, and it might be due to inhibition or stimulation of immune responses naturally. Probiotics are considered to affect the inequity on production of cytokines which is known to affect inflammation in RA patients. Cell-to-cell communication is observed in case of cytokines for immune responses in innate and acquired immunity (Gill and Prasad 2008). The patients with RA suffer from an overproduction and inadequate production especially of pro-inflammatory cytokines as well as anti-inflammatory cytokines, respectively. Table 13.1 summarizes the list of probiotic strains.

Probiotics stimulate the production of some cytokines, especially gene clusters of interleukin (Gill and Prasad 2008). Probiotics provide significant effect on the immune system, which might be explored for an interference to treat RA. Results or outcomes obtained from probiotic diet treatment differ among different population because of variation in strains of bacteria, dose as well as treatment rate. Daily consumption should be minimum 10^9 colony forming units (CFUs), while the optimal dose of each strain should be optimised for better outcomes. The probiotics generally counteract with minimal side effects like nausea, thirst and bloating but with a wider safety margin (Mohammed et al. 2017). Probiotics are easily accessible and widely available across the globe and mostly found in food, in the form of cheese, yogurt, fermented fish, meats and vegetables (Anadón et al. 2016) and also in the form of food supplement in the form of pharmaceutical form as tablets, powders, pills, capsules, liquid concentrates in soft gels and vials, (Anadón et al. 2010).

Table 13.1 Most commonly used species of bacteria and their strains as probiotics (Kim et al. 2015)

S. No.	Species	Strain
1.	*Lactobacillus*	*Lactobacillus johnsonii* LA1
		Lactobacillus rhamnosus GG
		Lactobacillus casei/Shirota
		Lactobacillus acidophilus
		Lactobacillus acidophilus LA5
		Lactobacillus acidophilus NFCM
		Lactobacillus gasseri
		Lactobacillus lactis
		Lactobacillus plantarum
		Lactobacillus reuteri
		Lactobacillus bulgaricus
2.	*Bifidobacterium*	*Bifidobacterium longum*
		Bifidobacterium bifidum
		Bifidobacterium animalis
		Bifidobacterium infantis
		Bifidobacterium breve
		Bifidobacterium adolescentis
3.	Others	*Enterococcus faecalis*
		Lactococcus lactis
		Clostridium butyricum
		Bacillus cereus
		Streptococcus thermophilus
		Enterococcus faecalis

Gupta and Singh (2011) provided criteria for microorganisms to be incorporated into group of probiotics which are as follows:

1. Survival on passage via GIT, i.e. at low pH along with bile.
2. Linkage with intestinal epithelial cells.
3. Equilibrium with intestinal microflora.
4. Pathogenicity must be zero.
5. Persistence in foodstuffs with prospectives in production of lyophilized preparations.
6. Proliferation must be fast and must have temporary or permanent colonization in GIT.
7. Probiotics have generic specificity.

13.3 Criteria for Selection of Probiotics

Probiotics are live microorganisms and directed in passable amounts, producing health effects within the host while prebiotics are food elements which are non-digestible which stimulates the progress with probiotics having activity (Fao

et al. 2002; Daliri and Lee 2015). Interestingly, it has been observed that some of non-living cells might also contain properties similar to probiotics (Guo et al. 2011; Bordoni et al. 2013). Stomach has extremely acidic nature because of the presence of hydrochloric acid. Probiotics must cross the primary barrier of gastric acidity and bile present in the upper GIT before entering into small intestines (Daliri and Lee 2015). The bacterial group exhibiting properties of probiotics are *Bifidobacteria* as well as lactic acid bacteria (LAB). *L. casei* as well as *L. acidophilus* both show their viability in acidic conditions simulated to gastric juice in pH 3.0 maintained at 37 °C, while *Bulgaricus, Lactobacillus delbrueckii* are not able to survive these conditions. Specific strains of *Bifidobacterium* have ability to survive in transit phase through the stomach. Table 13.2 summarizes the properties and advantages encountered with probiotic strains.

Probiotics selection and initial screening is based on below mentioned factors:

1. Utilization patterns of protein and carbohydrate.
2. Stability testing of phenotypic and genotypic traits which includes stability of plasmids; determining adhesiveness with intestinal epithelium.
3. Resistance patterns against antibiotics; inhibition of pathogens or organisms of spoilage type or both.
4. Generation of substances with antimicrobial properties.
5. Potency of immunogenicity.

The probiotic strains should be live, multiply and colonize their specific locations. Probiotic strains act as an adjuvant, and stimulates immune response against pathogens. Probiotics must be easily culturable for commercialization purposes in large quantities and able to withstand the fluctuation in heating as well as low oxygen conditions in packages.

Table 13.2 Advantages and properties of probiotic strains

Properties	Advantages
Resistance to bile acids and pancreatic enzymes	Viability in route through the gastro-intestinal tract
Adherence with intestinal epithelia/mucosa	Immune variation; exclusion of pathogen; benefit to repairing of damaged mucosa; continuation in transient occupation
Human evolution	Health benefits highly dependent on species and perseverance of feasibility
Genesis of substrates similar to antimicrobial	Act as an antagonist against pathogenic organisms
Predicted healthy outcomes	Predicted health outcomes are “true”; which are clinically authorized as well as have minimum active dose in products
Health	“GRAS” strain have “history of safe use”
Good knowledge properties	Consistency in strain; uninterrupted production; with tolerance to oxygen

13.4 Mechanism of Action of Probiotics

Evidence about the definite mechanism of probiotics is not understood clearly. But, some insights have been obtained from certain animal models as well as in vivo experimentation. A probable mechanism of action about probiotics is to act on strengthening of gut mucosa barrier functions. Most strains of *Bifidobacterium* and *Lactobacillus* stimulate signalling pathways of epithelial cell via their structural components and microbial-formed metabolites (Daliri and Lee 2015). It has been found that probiotics modulate several important pathways like Nuclear Factor Kappa-Light-Chain-Enhancer of activated B cells (NF-kB) and observable effects on I Kappa B protein (IKB) (Thomas and Versalovic 2010), proteasome function (Shiou et al. 2013) and Re1A nuclear-cytoplasmic movement by using PPAR-gamma pathway. *Streptococcus thermophilus* and *Lactobacillus acidophilus* are some of the probiotics with alteration in expression to tight junction proteins explained via both in vitro as well as in vivo models (Resta-Lenert and Barrett 2003). The alteration in expression levels for tubulin gene coding, occludin, cytoskeleton anchoring proteins and proteasome is shown in *Lactobacillus plantarum* MB452 (Anderson et al. 2010). The cytokine prevention and oxidant-induced damage for epithelial by promotion of cell survival is achieved by probiotics (Liu et al. 2015). Probiotics regulate the functions of immune system. For example, *Lactobacillus acidophilus* reported to modulate with toll-like receptors as well as recognizing enterocytes proteoglycan proteins and leads to dendritic cells initiation along with response from lymphocytic T-helper 1. Suppressing responses of lymphocyte T-helper 2 provokes the atopic issues and results in stimulating cytokines from lymphocytes T-helper 1 (Cosmi et al. 2014).

Above discussed mechanism explains the usefulness of administration of probiotics like *Lactobacillus acidophilus* and L. *rhamnosus* GG, especially in cases like children's skin sensitivity as well as disorders like eczema (Wickens et al. 2008; West et al. 2009). Probiotics suppress the pathogenic bacteria growth with the generation of bacteriocins which are broad spectrum (Hardy et al. 2013). The ability of colonization is enhanced by *lactobacilli* strains and expression of human mucus-binding pili (Turroni et al. 2013). Some of the probiotics inhibit the binding of pathogens to gut wall with genesis of short-chain fatty acids (SFCA) and results in reduction of gut pH for selectively facilitate the development of suitable microbes. Examples of such probiotics are *L. acidophilus* MB 443, *B. infantis* Y1, *L. paracasei* MB 451, *L. plantarum* MB 452, *L. bulgaricus* MB453 (Park et al. 2019). The intake of probiotics affects various aspects related to nonspecific innate immune systems including promoting production of mucin, inhibiting pathogenic type of bacteria, lowering of gut permeation, macrophage stimulation, natural killer (NK) cell activity and phagocytic capacity. Increased production of antibodies especially IgM, IgA and IgG and modulating immune system branches mediate production of regulatory elements and cytokines. Table 13.3 and Fig. 13.1 briefly summarize probiotic mechanism of action. The genetically alteration of microorganisms of second-generation probiotics offers the host and including some essential components like production of immune-modulators, such as

Table 13.3 Various mechanism of action in relation to probiotics

S. no.	Mechanism of action	References
1	Increase in competition for nutrients with pathogenic bacteria as well as for sites of adhesion and thus reducing survival rate of pathogenic bacteria	Fooks and Gibson (2002)
2	Production of bacteriocins which are antimicrobial materials and dangerous for pathogenic bacteria	Gibson and Wang (1994)
3	Producing short-chain fatty acids with the help of fermentation of carbohydrates. Fermented carbohydrates may lead to: (a) Supplying nutrition to colonocytes (b) Decreasing the pH of the colon ultimately destructive for pathogenic bacteria (c) Changing gene expression of epithelial (mostly an effect of butyrate)	Fooks and Gibson (2002), Sanderson (2007)
4	Decreasing intestinal permeability	Rosenfeldt et al. (2004)
5	Modification of immune functionality via direct interaction with mucosal associated immune system	Wang et al. (2016)

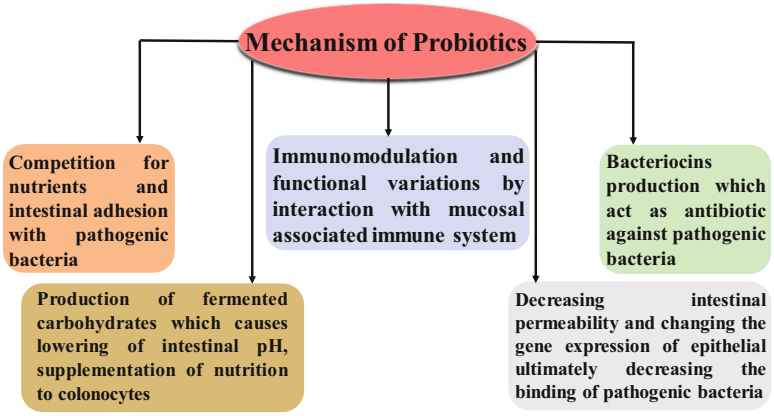

Fig. 13.1 Summarization of probiotic mechanism of action

interleukins. (Mercenier et al. 2005). The detailed selection of a specific probiotic strain for targeting of patient's specific pathogen deficiency and clinical problem may help in future probiotic-specific mechanisms (Ciorba 2012).

13.5 Rheumatoid Arthritis

Rheumatoid arthritis (RA) is affecting 0.5–1% of adult populations with chronic and systemic autoimmune inflammatory disorder and characterized by chronic pain of synovial joint, bone destructions and progression in disability (Scarno et al. 2014). It can be quickly damaging, with almost 60% of RA patients suffers with erosions of

joints when investigated with radiographs within 2 years of onset of disease (Ranganath and Furst 2007). The patients with RA have to suffer from functional ability loss and lead to disability and act as an economic burden to society (Ranganath and Furst 2007). Till 1953, patients reported with RA have premature mortality with an approximate of 10 year (Cobb et al. 1953).

In the USA, more than 54.4 million men and women are reported to be affected by RA (Vitaliti et al. 2014). RA predominantly inflicts the hands joints, wrists and knees. Disease modified non-steroidal anti-inflammatory agents (NSAIDs), anti-rheumatic drugs (DMARDs), corticosteroids, self-managing strategies and biological response modifiers are the current treatment options for RA (Vitaliti et al. 2014). Aetiology associated with RA disease is still unknown, and this treatment simply manages the disease. In the development of RA various genetic factors are employed (Bedaiwi and Inman 2014). RA is extensively studied and explored among any autoimmune disorders in regard to microbial dysbiosis. After conducting several studies consider the monozygotic as well as dizygotic twins, with lowering in percentage of concordance in the prognosis of RA (Bedaiwi and Inman 2014). Several studies have proved that there might be an environmental factor which causes autoimmune responses in genetically susceptible individuals (Sandhya et al. 2016). Abnormal immune function with excessive production of autoantibodies followed by pro-inflammatory T lymphocytes production are the hallmarks of RA (Jethwa and Abraham 2017). However, several studies report if any alteration exists in gut microbiota has close relation with chronic stages of RA (Scher and Abramson 2011; Kamada et al. 2013; Kim et al. 2015), which states that, administering certain probiotics might suppress in experimental RA patients (Kato et al. 1998) (So et al. 2008a). The patients examined with inflammatory type of arthritis are observed with elevated antibodies to antigens ratio (Tiwana et al. 1998; Brandtzaeg 2006), which leads to deposition of immune complexes in joint capsule supplied through capillaries (Angeles et al. 2011). Oxidative stress, inflammation and insulin resistance play a major role in RA pathogenesis (Geronikaki and Gavalas 2006; Maruotti and Cantatore 2015). RA is also related with increased risk of mortality and morbidity from cardiovascular diseases (CVD) (Paccou et al. 2012) along with type 2 diabetes mellitus (T2DM) (Wasko et al. 2011). Many microbiomes are linked to prognosis of arthritis and its site is summarized in Table 13.4.

13.6 Probiotics in Rheumatoid Arthritis

Many studies of randomized controlled clinical trials (RCTs) assessed effect of diversity in probiotic strains and association with RA patients. The activity of disease, functional improvement and status of inflammation in RA patients on receiving the treatment of probiotics have been reported in many studies (Hatakka et al. 2003; Mandel et al. 2010; Angeles et al. 2011; Alipour et al. 2014; Vaghef-Mehrabany et al. 2014).

Probiotics provide an immune-regulation rather than immune-activation. Probiotics directly control immune responses and sustain the gut immune

Table 13.4 Microbiome linked to Arthritis and presence on site

Bacterial species	Site	Reference
Prevotella intermedia	Oral	Moen et al. (2005), Martinez-Martinez et al. (2009)
Porphyromonas gingivalis	Oral	
Flagellin	Serum	Van Praet et al. (2014)
Prevotella copri	Faecal	Bernard (2014)
Streptococcus	Lung	Willis et al. (2013)
Haemophilus	Lung	
Streptococcus anginosus	Supragingival	Wolff et al. (2014)
Tannerella forsythia	Subgingival	

homeostasis along with improvement in epithelial barrier function, while constraining growth of pathogen (Kirjavainen et al. 1998), thereby exerting pathogenesis of RA (Wang et al. 2016). Leflunomide, hydroxychloroquine and methotrexate which come under the category of disease modifying anti-rheumatic drugs (DMARDs) reduce inflammation due to slow RA progression (Eter et al. 2000; Li et al. 2016; Sharma et al. 2016). But side-effects related with DMARDs cannot be ignored. Various studies have indicated that diet is highly associated in management of RA patients. The probiotic like *L. rhamnosus* GG (LGG) is a short-time probiotic therapy with potential for reinforcing the mucosal barrier in case of juvenile chronic arthritis (Ni et al. 1997). Naturally containing high amounts of lactic acid bacteria (LAB) in vegan diet is reported to reduce RA severity as well as provide relief from the symptoms (Peltonen et al. 1994). Different probiotics contain different types of microorganisms especially one of the largest genus which contain more than 50 species, i.e. LAB. *Bifidobacterium* and *Lactobacillus* are LAB probiotics (Argyri et al. 2013), and their existence is in human intestine with an advantage of non-pathogenic nature (Wang et al. 2016). They produce diacetyl acetaldehyde, organic acids, bacteriostatic element or other factors which inhibit the progressive degeneration in organisms, decreasing toxin, regulating the immune system and promotion of bowel movement (Du Toit et al. 1998). Microorganisms are gaining medical attention because of their antagonistic effects and therapeutic activity against human pathogens. Probiotic LAB inhibits the growth of many Gram-positive as well as Gram-negative pathogenic bacteria, such as *Salmonella typhimurium, Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis* (Tejero-Sariñena et al. 2012). Table 13.5 summarizes clinical trials carried out for rheumatoid arthritis by using probiotics.

RA patients undergo long-term therapy of pharmaceutical drugs which although is effective but produce unpleasant side-effects. Complementary and alternative medicine (CAM) is prescribed in above 30–60% of RA patients for relief of pain (Fernández-Llanio Comella et al. 2016). Probiotics may provide adjuvant therapy to RA as nutrient supplement. A limited number of positive relationships are reported linking administration of different probiotic strains orally and correlation with RA activity in studies with murine models and human subjects, and these

Table 13.5 Brief summary of clinical trials of probiotics in management of rheumatoid arthritis

S. no.	Probiotic strain	Study design	Sample size/ duration of therapy	Results	References
1.	*Lactobacillus rhamnosus* GG	Placebo-controlled, double-blind randomized	21/ 52 weeks	No statistical differences was observed in the biochemical variables, clinical parameters with Health Assessment Questionnaire (HAQ) index	Hatakka et al. (2003)
2.	*Bacillus coagulans*	Placebo-controlled, double-blind randomized	45/ 60 days	Treatment causes reduction of C-Reactive Protein (CRP)	Mandel et al. (2010)
3.	*Lactobacillus rhamnosus* GR-1 and *lactobacillus reuteri* RC-14	Placebo-controlled, double-blind randomized	29/ 14 weeks	Clinically not much progress but on individual reports mild progress was observed on comparison with placebo controlled	Angeles et al. (2011)
4.	*Lactobacillus casei* 01	Placebo-controlled, double-blind randomized	46/12	Average results were obtained but not of significant difference	Alipour et al. (2014)
5.	*Lactobacillus casei 01*	Placebo-controlled, double-blind randomized	46/8	Marked difference was observed in comparison to Placebo	Vaghef-Mehrabany et al. (2014)

studies show different results with different probiotic strains and dose (Dhanoa 2019). When probiotics were administered orally in animal models there is reduction in severity of arthritis (Kano et al. 2002; Rovenský et al. 2009, 2005). The probiotics' usage is for prevention or treatment of arthritis largely remained unexplored however, they can be employed as adjuvant therapy in case of RA.

The following section describes some examples of the clinically relevant probiotics.

13.6.1 *Lactobacillus*

Lactobacillus is used to treat various ailments associated GIT and urogenital disease. Many strains of *Lactobacillus* have probiotic property. Probiotics might modulate

the cytokine production and thus suppress the inflammatory responses (Gibson and Wang 1994; Sanderson 2007). *In vitro* evaluation of *Lactobacilli* predicted upregulation of cytokine expression especially in murine dendritic cells (DCs) as well as human peripheral blood mononuclear cells (PBMC) (Braat et al. 2004; Christensen et al. 2002; Matsuguchi et al. 2003; Miettinen et al. 1996). Several studies considered that *Lactobacillus* species maintain and regulate intestinal homeostasis and are harmless (Vitetta et al. 2013). Probiotic strains obtained from different sources induce immune-activation through various signalling pathways and lead to production of certain immune responses like IL-12, T helper 1 (Th1) cytokines, tumour necrosis factor (TNF)- α, IL-2 and IL-1β (Bunout et al. 2002) and PBMC which contain IL-18 (Miettinen et al. 1996, 1998; Maassen et al. 2000; Christensen et al. 2002) and found to activate nuclear factor toll-like receptors (TLRs), κ-light-chain-enhancer of activated B (NF-κB) (Miettinen et al. 2000; Matsuguchi et al. 2003; Rachmilewitz et al. 2004). Recently some authors reported that certain strains of *Lactobacillus* species have potential anti-inflammatory as well as anti-cancer activities.

13.6.2 *Lactobacillus rhamnosus*

Lactobacillus rhamnosus GG (LGG) is probiotic bacteria. The probiotic treatment with LGG for cure of RA did not produce clinically substantial benefits though more than 71% of LGG group patients were registered with better treatment outcome (Hatakka et al. 2003) with no serious effects. It also has viability for the strong acids which is found in stomach. Evaluation of effect of LGG was performed in RA patients as pilot study (Hatakka et al. 2003). In this study 21 RA patients were selected for randomization according to probiotic group. Assessment of arthritic activity for this study includes health assessment questionnaire (HAQ) index, clinical examination as well as laboratory tests for both placebo and probiotic group. Significant reduction in tender or swollen joints was reported on treating with the *Lactobacillus* treated group (8.3–4.6%) in comparison to placebo group (5.5–4.8%).

Oral administration of *L. rhamnosus* by Nowak et al. (2012) reported distinct types of experimental arthritis. *L. rhamnosus* KL37 derivative of *L. rhamnosus* was shown to depresse the anti-collagen IgG production and affected the growth in mice via collagen induction. From above observations a general compilation states that *L. rhamnosus* GR-1 and LGG might act by decreasing the production, especially of granulocyte-colony-stimulating factor (G-CSF) (Kim et al. 2006). Downregulation of pro-inflammatory is followed by upregulation of anti-inflammatory cytokines governed by *L. rhamnosus* in human placental trophoblast cells (Yeganegi et al. 2011).

13.6.3 *Lactobacillus casei*

Lactobacillus casei (*L. casei*) is harmless probiotic, non-pathogenic in nature, and regulates immune function of both animal and human (Matsumoto et al. 2005; Agüero et al. 2006; Van Baarlen et al. 2011). Supplementation with *L. casei* provided improvement in RA symptoms as well as various inflammatory biomarkers (So et al. 2008b; Alipour et al. 2014). *L. casei* 01, reported to be a subspecies of *L. casei*, exerted its effects by adhesion with intestinal epithelia and resistance to gut environment stimulation (Chan et al. 2010) with intestinal adhesion due to Caco-2 and (IECs)-6 cells, an essential indicator for microorganism colonization (Tuomola and Salminen 1998). *Eubacterium aerofaciens* and *L. casei* result in the genesis of pro-inflammatory cytokines and used for inducing arthritis in rats (Šimelyte et al. 2000). Randomized double-blind clinical trials were performed for confirming whether *L. casei* is progressively participating in prognosis of disease or not (Alipour et al. 2014). In this study, 22 female receiving one capsule containing *L. casei* 01and 24 female patients as placebo groups, respectively. Disease activity score-28 (DA28) was calculated according to European League Against Rheumatism (EULAR) and response was evaluated during the intervention period, and estimation of cytokines, IL-10, IL-1β, IL-12, IL-6 and TNF-α. The above results on compilation showed that supplementation of *L. casei* 01 capsule affected both inflammatory cytokines and disease activity. The results showed that the factors such as CRP levels were lowering in probiotic group. Based on EULAR criteria, patients reported weakened response to the treatment at the end of study.

Vaghef-Mehrabany et al. also performed the randomized double-blind clinical trial and showed comparable findings that supplementation of *L. casei* 01 results in a reduction of disease activity in patients with RA. Another study reported that *L. casei* could be considered as an effective nutraceutically derived modulator for management of osteoarthritis by decreasing the inflammatory response arising due to pain and degradation of articular cartilage (Anastasiou et al. 2014). It was also observed that combination of *L. casei* along with type II collagen (C II) and glucosamine (Gln) might be more effective in reducing pain, cartilage destruction and lymphocyte infiltration other than oral administration of glucosamine or *L. casei*. Various pro-inflammatory cytokines were affected by this co-administration, downregulating IL-1β, IL-6, IL-6, IL-12, IL-2, IL-17, TNF-α and IFN (interferon)-γ.

13.6.4 *Lactobacillus plantarum*

Gohil et al. studied complete Freund's adjuvant (CFA)-induced arthritis in rats and observed reduction in movement of inflammation due to content of cell wall of *L. plantarum*. Studies were divided into six groups and performed on female Wistar rats by creating a model of chronic polyarthritis. In treatment groups, rats were administered standard dexamethasone or any one of three dosages containing cell wall content of *L. plantarum*: 10^5, 10^7, 10^9 CFU/animal. Induction of arthritis in animals was verified by physical measurement of paw volume, joint inflammation,

body weight, lesions, gait and mobility. The biochemical evaluation includes; C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), serum TNF-α and rheumatoid factor (RF). Improvement in above factors was shown by CFA-injected rats receiving treatment with *L. plantarum,* suggesting probiotics have antiarthritic activity (Gohil et al. 2018). Highest dose of 10^9 CFU/animal of *L. plantarum* predicted promising results.

13.6.5 *Lactobacillus reuteri*

Lactobacillus reuteri (*L. reuteri*) is an another subtype probiotic which is efficient in treatment of immature colon (Savino et al. 2007), severe diarrhoea, rotavirus, *Gardnerella vaginalis* (Anastasiou et al. 2014) and also in relieving from the frequent eczema occurrences (Abrahamsson et al. 2007). The destruction and infection in relation to *Helicobacter pylori* (*H. pylori)* were decreased by *L. reuteri*. *L. reuteri* has been regarded as safe with its use from past many years with promising supplementary probiotic diet in adults. In human GIT *L. reuteri* was found to be an endogenous to *Lactobacillus* species. Recent studies showed that *L. reuteri* is safe in new born infants when used as dietary supplements for long intervals (Connolly et al. 2005). In a study, both *L. reuteri* RC-14 and *L. rhamnosus* GR-1 were administered orally as probiotics or placebo group and randomly organized in 29 RA patients (Angeles et al. 2011). Parameters like, American College of Rheumatology criteria for 20% improvement (ACR20) responses along with clinical examination, laboratory tests, serum cytokines levels were evaluated. Laboratory tests showed substantial lower levels of IL-12p70, IL-8 and macrophage inflammatory protein (MIP)-1β comparable in the probiotic group with placebo group. ACR20 response observed in both groups was not significantly different. Similarly it was observed in case of IL-6, IL-15, granulocyte/macrophage colony-stimulating factor (GM-CSF), IL-1α, and TNF-α levels were not significantly different among the two groups.

13.6.6 *Lactobacillus helveticus*

Lactobacillus helveticus (*L. helveticus*) also reduces activity of RA disease by same mechanisms as discussed previously (So et al. 2008b; Alipour et al. 2014). The study by Kim et al. significantly proved by utilizing ex vivo screening system prior to in vivo studies in mice. Selection of suitable strain for probiotic will be best for treating collagen induced arthritis (CIA). The process of screening was performed using culturing lymphocytes obtained from the lymph nodes along with probiotics. Based on the low expression profile of IL-10/high/IL-12p *L. helveticus* HY7801 was

selected. The ratio of anti-inflammatory/pro-inflammatory cytokines was selected for its further implications in progression of RA development as it could make a major selection marker to choose probiotic.

Outcomes of the study concluded that supplementation with probiotics for 3 weeks earlier to initiation of CIA provides a preventive effect for the development of CIA (Kim et al. 2006). The study is performed on three strains which were considered as pre-treatment options: *L. helveticus*, *Bifidobacterium longum* and *Lactobacillus johnsonii*. Administration of dose to mice was 5×10^8 CFU/day. The results observed that all three recommended probiotics had significantly reduces CIA development; but, *L. helveticus* HY7801 was found to be significantly reducing CII-reactive immunoglobulin antibodies. Selection of above discussed strain was continued for treatment of arthritis during the experiment's second phase. In later studies related to *L. helveticus HY7801,* reduction in paw swelling was observed in experimental RA murine model. By these studies it was showed that *L. helveticus* had decline disease activity and decreases pro-inflammatory cytokines like IFN-γ, TNF-α and IL-17A and enhancing expression of IL-10 by CD4T cells (Kim et al. 2006). These studies were correlating with another study (So et al. 2008b) and demonstrated the capability of probiotics in treatment of experimental RA and transforming into human beneficial effects.

13.6.7 *Lactobacillus brevis* and *Lactobacillus plantarum*

Although probiotics are employed as supplementary and used for the therapy of RA, along with efficient foods may also be employed. Nenonen et al. (1998) studied therapeutic efficacy and correlated uncooked, vegan diet, lactobacilli-rich in RA patients. A larger amount of probiotics, chlorophyll and fibre are provided by this diet. A decrease in symptoms associated with rheumatic pain, like morning stiffness and joint swelling in RA patients who were feeded with living food in comparison to control group maintained on diet equivalent to an omnivorous diet. There was no statistical significant difference observed among both groups in number of tender joints and swollen. It was shown that significant decrease of RA activity was associated with consumption of fermented drinks and diet rich in iron and fibre. Rich source of *Lactobacilli* strains is fermented wheat drink and contains $2.4–4.5 \times 0^{10}$/day of *Lactobacillus brevis (L. brevis)* and *L. plantarum* and was given to the experimental group patients which exhibited an increase in faecal lactobacilli by analysis of faecal microbiota. Nenonen et al. noticed the declining in RA activity in one of the experimental group and observable diet-induced changes in intestinal microflora of patients. The half of the patients stopped the treatment due to adverse effects like nausea and diarrhoea which suggest that high amount of diet therapy is not advisable in all cases of patients. But vegan diet which is rich in lactobacilli might be therapeutic beneficial in RA measures.

13.6.8 *Lactobacillus rhamnosus* and *Lactobacillus reuteri*

There was no significant improvement in clinical criteria for RA when administered orally to *Lactobacillus rhamnosus* GR-1 as well as *Lactobacillus reuteri* RS-14. But the significant difference was observed between experimental group and control group (Angeles et al. 2011). Pineda et al. performed pilot study on patients with severe type of RA as well as chronic synovitis. Dose of 2×10^{10} CFU was administered twice a day and given to probiotic group patients (Angeles et al. 2011). Main outcomes of the study were to achieve an ACR20 response in patients on probiotic therapy in comparative to placebo group. Another method for measuring the arthritic activity was to physically measure the swollen and tender joints, HAQ, morning stiffness, patient's pain assessment and physical global assessment. The biochemical parameters like CRP, ESR and 15 inflammatory cytokines (IL-1β, IL-6, IL-8, IL-12p70, TNF-α, IL-15, IL-10, GM-CSF, G-CSF, IL-17, Scd40 ligand, MIP-1α, MIP-1β, and MCP-1) were included. No statistically difference was observed on the basis of main outcomes.

Patients administered with probiotic group reported to significantly increase in HAQ scores on comparing with baseline when observed statistically (Angeles et al. 2011). The results obtained in above-mentioned studies correlated with another study where clinical improvements were not so much significant (Hatakka et al. 2003). HAQ might be better than other clinical evaluations to compare illness and functionality in RA patients. This remains to be verified, therefore, the effect of *Lactobacillus reuteri* and *Lactobacillus rhamnosus* as an adjuvant in RA treatment is difficult to conclude. Size of the sample for study was too small with total size of 29 only and probiotic receiving group was limited to 15 only to assess ACR 20 response. Further thorough studies are required to properly determine whether the probiotics have the chances of improving functionality in the long term.

13.6.9 *Lactobacillus casei*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum*

Probiotic treatment combined with *Lactobacillus casei*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum* was found promising in counteracting RA patients. In 2016, Zamani et al. designed randomized control trials for testing multispecies probiotic effects and using 6×10^9 CFU/g as total dose. Individual strains of bacteria were assessed at 2×10^9 CFU/g. The standard arthritis medication was continued to the patients of both the treatment as well as placebo groups. Patient's arthritis was measured primarily by inflammatory factors and the disease activity score of 28 joints (DAS28).

13.6.10 *Bacillus coagulans*

Bacillus activates intestinal gut microflora, has resistance to low pH and basically belongs to LAB strain. *Bacillus* also shows similar effect to *Lactobacillus* for improving quality in RA patients.

Parallel design double-blind randomized and placebo-controlled study was performed by Mandel et al. (2010) for studying an intervention in humans. Results proved that patients receiving *Bacillus coagulans* experienced small incremental benefit, i.e. statistically significant but at borderline in comparison to placebo in terms of pain scale. By this study it was found that *Bacillus coagulans* probiotic was safe and showed effective treatment for RA patients. As the study was conducted on small number of samples, a larger sample size is needed to verify these results.

Abhari et al. (2016) used an in vivo model of male Wistar rats induced with CFA. In this study investigation of therapeutic effects of probiotic, prebiotic as well as symbiotic diet with inflammatory markers of RA was observed. The results obtained from above study were in comparison to control group which was administered indomethacin as a reference. In case of groups receiving the probiotic therapy, rats were administered with *B. coagulans* with dose of 10^9 spores/day. Parameters for measurement of arthritis were paw thickness, fibrinogen (Fn), TNF-α, alpha-1-acid glycoprotein (α1AGp) and serum amyloid A (SAA). In RA critical autoantigen is Fn. SAA is known to activate pro-inflammatory Th 1 cells which regulate the angiogenesis behaviour, leukocytes as well as matrix degradation. Fn, TNF-α and SAA have significant effect in RA joint damage. The results showed statistically significant improvement in the treatment group (Abhari et al. 2016).

The production of Fn, SAA and TNF-α pro-inflammatory cytokines was decreased in *B. coagulans* group. In this group significant decrease in paw thickness was observed (Abhari et al. 2016). Supplementary administration of *B. coagulans* in RA treatment shows its effects via mechanisms like prostaglandin downregulation by anti-inflammatory cytokines.

13.7 Conclusions

Till date no extensive studies were performed which states the exact role of probiotics in treatment of RA. As per the reported literature studies, *Lactobacillus* and its strains had shown promising results in clinical trials for therapeutic intervention in rheumatoid arthritis for human usage. The most lacking component of these studies is that the mechanism of probiotics for management of rheumatoid arthritis is still uncertain. The futuristic challenge about these strains is to understand how they interact with microbial biofilms present in the gut and intestine and how they revive the natural flora of gut. Although NSAIDs, DMARDs and biologicals are used in treatment of RA, probiotics showed an extensive effect on RA patients with no observation of adverse effects clinically. Probiotics are administered along with foods so that they can be patent compliant. Probiotics can be proposed as an adjuvant therapy which is beneficial in treatment of RA patients but it might also highlights

other new strategies with chronic, systemic, and autoimmune inflammatory disease. Therefore, the probiotic therapy in RA treatment with positive effects can be regarded as safe, effective and most convenient option therapeutically. However, for better understanding further studies are required to explore the anti-inflammatory properties of probiotics in the RA treatment.

Conflict of Interest The authors declare no conflict of interest among themselves.

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Genetically Engineered Probiotics

14

Saba Haq and Naresh Poondla

Abstract

Continuous rise in the antibiotic-resistant microbes coupled with sluggish antibiotic development procedures have paved ways for alternative means to curb diseases. The advancement in the field of synthetic biology, genetic engineering, and genome sequencing technologies has led to implementation of the notion pertaining to improving the functional repertoire of prevailing microbes. The sophisticated approach of using genetically engineered probiotic/designer probiotics is based on altering the genetic makeup of probiotic strains for improving the health management, livestock, and aquaculture industries. Designer probiotics are tailored for expressing beneficial protein, biomaterial delivery, destroying infectious pathogens to combat cancers, infectious, and metabolic diseases. In addition, they exhibit applications in improving animal feeding consumptions, survival, body weight, and growth rates thereby leading to health and financial gains. Herein, we have focused on the current progress in the field of designer probiotics, safety concerns regarding their practical applications and discussed the potential prospects for their clinical translation.

Keywords

Designer probiotics · Infection · Metabolic diseases · Cancer · Inflammation

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S. Haq
Hanyang University, Seoul, South Korea
e-mail: sabahq1@hanyang.ac.kr

N. Poondla (✉)
Department of Clinical Research, Richmond University Medical Center, Staten Island, NY, USA
e-mail: NPoondla@rumcsi.org

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14.1 Introduction

The term patho-biotechnology was coined to refer to the exploitation of pathogenic microbes for beneficial utilization in food industry as well as biomedicine (Sheehan et al. 2006). The field of patho-biotechnology involves three distinct approaches (Fig. 14.1); the foremost approach comprises of utilizing attenuated bacterial pathogens in vaccine or drug delivery mechanisms (Wright and Roland 2005). Another approach focusses on the direct application of pathogen-related immunogenic proteins, while the last approach of the field of patho-biotechnology deals with equipping the probiotics with the stress survival approaches. It involves modifying the probiotic/non-pathogenic microbes with the genetic constructs to overcome issues during food production, storage conditions and also during the body defense mechanism such as gastric acidity, bile, etc. The aforementioned third aspect of patho-biotechnology further encompasses three strategies. First one deals with the storage and delivery of the probiotics by cloning as well as expression of pathogen-related stress survival machinery thereby facilitating enhanced survival of probiotics at harsh temperature and water conditions. The second one tackles with the augmentation of host colonization by expressing host specific survival mechanisms. The last one encompasses the development of recombinant probiotics usually referred as

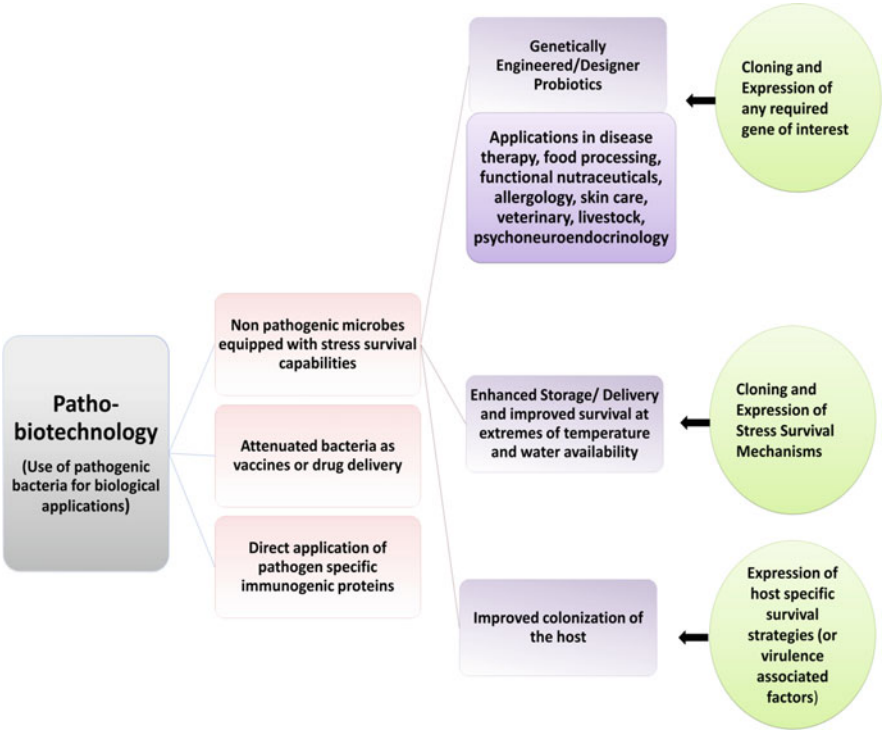

Fig. 14.1 Overview of the concept of patho-biotechnology

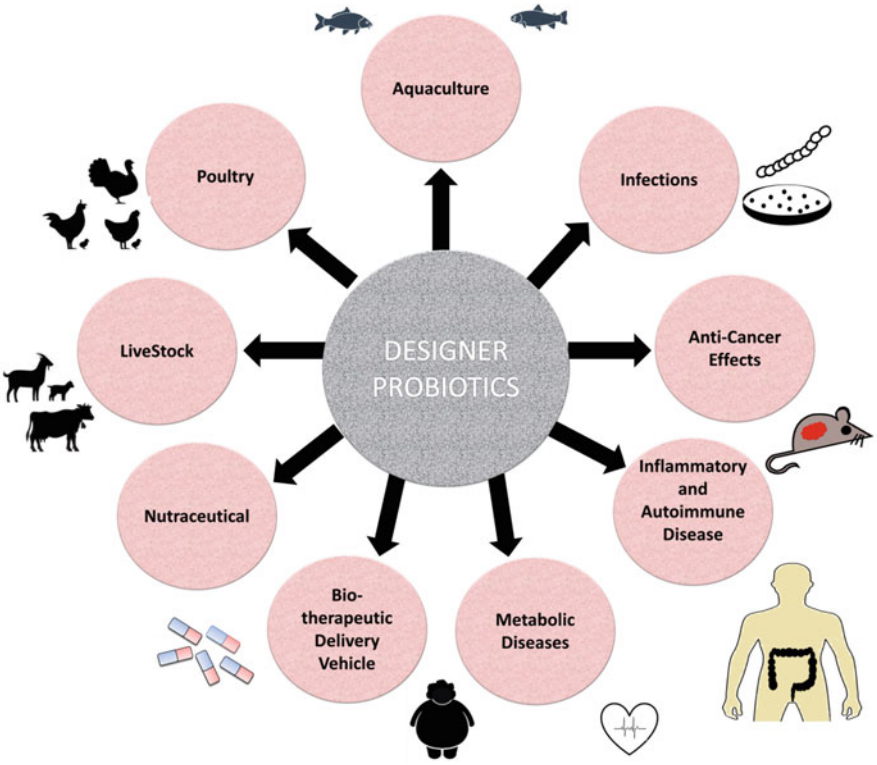

Fig. 14.2 Applications of bioengineered probiotics

"Designer Probiotics/Probiotics 2.0" to specifically target invading pathogens via blockage of critical receptor–ligand interaction between the host cell and the pathogen (Sleator and Hill 2008). In the current postgenomic era of medicine and biotechnology, the applications of probiotics, though previously restricted to food processing and general microbiology, have now expanded towards the fields of oncotherapy, infectious diseases, functional nutraceuticals, allergology, skin care, veterinary, livestock, psycho neuroendocrinology, and gastroenterology (Fig. 14.2).

14.2 Designer Probiotics as Diseasomic Approach

The normal microbes though being associated with normal processing like aging, hematopoiesis, immunity, etc. lack the ability to protect host organisms against external pathogens, thus leading to various infections. Simultaneous increase in the antibiotic-resistant pathogens along with slow antibiotic development process has shifted the attention towards alternative means such as improving the functional repertoire of existing probiotics. Living therapeutics, generated through engineering

microbes, are efficient in fighting challenging medical problems such as chronic conditions, metabolic diseases, cancers, infectious and autoimmune diseases (Table 14.1). Such engineered probiotics can provide targeted in situ delivery of antitumor agents, while avoiding the damaging effects on healthy cells caused by systemic treatment. Molecular tools for engineering designer probiotics have increasingly become diverse with time, thereby enabling the design of sophisticated genetic circuits for targeted applications. Thus, the designed probiotics capable of site-specific delivery of therapeutics through genetic transformations may change existing paradigms of disease management (Braat et al. 2006; Paton et al. 2012; Kumar et al. 2016; Maxmen 2017).

14.3 Production and Targeted Delivery of Therapeutic Agent

Peptides, proteins, and small metabolites are used as therapeutic agents in engineered bacteria (Borrero et al. 2015; Jayaraman et al. 2017; Hwang et al. 2014). For metabolic diseases such as diabetes mellitus and intestinal diseases single-chain insulin analog, human proinsulin, and interleukin-10 (IL-10) are used as therapeutic molecules (Benbouziane et al. 2013; Takiishi et al. 2017). Introducing a secretion tag to the polypeptide chain is a common strategy for delivery purposes. For instance, the secretion tag usp45 and yebF tag have been widely employed in engineered *Lactococcus Lactis* (Borrero et al. 2015; Ng and Sarkar 2011; Shigemori et al. 2015; Mao et al. 2017) and *Escherichia coli* (Hwang et al. 2014; Ho et al. 2018), respectively. For the purpose of optimal growth, production, and subsequent release of the cargo, designer probiotics are engineered to control the expression of phage lysins to an internal (Hwang et al. 2017) or external (Camacho et al. 2016) input signal.

After its secretion or release, therapeutic agent still needs to reach the target effectively. Therefore, in order to overcome the limitations of bacterial diffusion, motile bacteria can be engineered to selectively swim toward its target (Hwang et al. 2014). Additionally, proteins can be displayed on the chassis surface to act as anchors (Marcobal et al. 2016) or to stimulate infiltration or adhesion on the target. For example, the ice nuclease protein (INP) tag on *E. coli* bacterium exports the polypeptide cargos to the membrane. Upon fusion with INP, histone-like protein A enhances infiltration of microbes into tumor for targeted therapy (Ho et al. 2018).

14.4 Designer Probiotics in Anti-Cancer Therapy

Cancer accounts for major deaths worldwide and the current conventional anti-cancer therapies fall short to completely eradicate and prevent its remission. In the past decade designed probiotics have appeared to aid the anti-cancer therapy as prodrugs, as diagnostic tools through improving the specific targeting, and so on. One application revolves around the ease of bacterial manipulation and the use of their colonizing ability in tumor-based hypoxic surroundings (Van Dessel et al.

Table 14.1 Applications of probiotics in medical industry against diseases

Therapy	Medical condition	Probiotic	Reference
Anti-cancer therapy	Hypoxic tumors	*B. longum*	Sasaki et al. (2006)
	Tumors	*E. coli* Nissle 1917	Brichacek et al. (2013)
	Hepatic metastasis	*E. coli* Nissle 1917	Danino et al. (2015)
	Colon carcinoma	*L. lactis* NZ9000-401	Zhang et al. (2016)
	Oral mucositis	*L. lactis*	Caluwaerts et al. (2010)
	Human colon adenocarcinoma	*L. lactis*	Bohlul et al. (2019)
	Metastatic cancer	*S. typhimurium* A1-R	Hoffman (2015), Hiroshima et al. (2015)
Inflammatory and autoimmune disease	Colitis	*B. longum*	Wei et al. 2016)
	Acute colitis	*B. ovatus*	Hamady (2013)
	Colitis	*L. lactis*	Steidler et al. (2000), Vandenbroucke et al. (2004)
	Crohn's disease	*L. lactis*	Braat et al. (2006)
	Inflammation of the GIT	*L. lactis*	Maddaloni et al. (2015)
	Inflammation	*L. lactis*	Del Carmen et al. (2014b), Shigemori and Shimosato (2017), Singh et al. (2017)
	Inflammatory bowel disease	*L. lactis* NZ9000 (NZ-HO)	Shigemori et al. (2015)
Infections	Streptococcal group A-mediated pharyngeal infections	*L. gasseri* NM713	Mansour and Abdelaziz (2016)
	Planktonic *Pseudomonas aeruginosa*-based infection	*E. coli*	Saeidi et al. (2011)
	Multidrug-resistant *E. faecalis's*	*L. lactis*	Borrero et al. (2015)
	HIV-1 based infection	*L. casei*	Pusch et al. (2006)
	HIV-1 based infection	LAB	Marcobal et al. (2016)
	Rotavirus-induced Diarrhea	*L. rhamnosus*	Álvarez et al. (2015)
	Bacterial Vaginosis	LACTIN-V	
	P. aeruginosa-mediated gut infection	*E. coli* Nissle 1917	Hwang et al. (2017)
	Intestinal *V. cholera*-based infection	*L. lactis*	Mao et al. (2018)

(continued)

Table 14.1 (continued)

Therapy	Medical condition	Probiotic	Reference
Metabolic diseases	Type 1 diabetes	*L. lactis*	Chamcha et al. (2015)
	Hyperglycemia	*L. lactis*	Liu et al. (2016)
	Diabetes	*L. lactis*	Robert et al. (2014)
	Diabetes	*L. lactis*	Duan et al. (2015)
	Diabetes	*B. longum*	Wei et al. (2015)
	Diabetes-induced harmful effects in retina and kidney	*L. paracasei*	Duan et al. (2015)
	Obesity	*E. coli* Nissle 1917	Khan et al. (2014)
	Hepatic Steatosis	*E. coli* Nissle 1917	Somabhai et al. (2016)
	Urolithiasis	*L. plantarum*	Paul et al. (2018)

2015). For instance, non-pathogenic bacteria like *E. coli* Nissle 1917 possess ability to target the tumors, as well as replicate adjacent to the tumors (Brichacek et al. 2013). Tumor colonizing facultative or obligate anaerobic bacteria, such as Shigella, Salmonella, few strains of *E. coli* or clostridia can be utilized in therapeutic measures against primary as well as metastatic cancers (Lee et al. 2005). Given the bacteria's capacity to proliferate preferentially in hypoxic core of solid tumors (Heap et al. 2014), presently supported by genome engineering as well as synthetic biology, designer probiotics may have applications as vectors for targeting tumor. Similarly, genetically engineered probiotics designed for producing tumor-targeting peptides result in tumor-specific cytotoxicity and provide remote cancer tissues with chemotherapy.

Salmonella typhimurium A1-R, a modified tumor-targeting probiotic, may substitute former therapeutic approaches and provide systemic treatment of metastatic cancer (Hoffman 2015; Hiroshima et al. 2015). Recombinant *L. lactis* NZ9000-401 is an example of an ideal probiotic organism to secrete bioactive tumor metastasis-inhibiting peptide named KiSS1. This is conceived as a plausible probiotic-mediated anti-cancer strategy by hindering Human colon carcinoma cells' proliferation as well as migration (Zhang et al. 2016). A stable secretory protein expressed in intestinal mucosa human trefoil factor 1 (hTFF1), is essential to repair epithelial damage in cancer patients occurring due to radiotherapy or chemotherapy-induced oral mucositis (OM). Administration of modified *L. lactis* strain secreting hTFF1 in a clinically relevant hamster model led to significant decrease in the severity and the course of oral mucositis. Thus, this methodology comprising of in situ secretion of hTFF1 through topical administration of *L. lactis* provided an efficient therapeutic method for not only the prevention but also treatment of radiation induced-OM complication (Caluwaerts et al. 2010). Utilizing designer bacterium to express anti-cancer compounds is another approach for fighting cancers. Engineered *L. lactis* expressing cell wall-anchored or secreted forms of human tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) protein induced apoptosis in the human colon adenocarcinoma cell lines (Bohlul et al. 2019).

Probiotics can be programmed to carry specific gene circuits that enable tumor detection thus can be considered as a diagnostic method. The probiotic bacterium *E. coli* Nissle 1917 (EcN), upon oral administration, can detect hepatic metastasis signals in patients' urine samples (Danino et al. 2015). Also recombinant *E. coli* have applications in sensing ribose sugar and glucose in solid tumor cell masses (Panteli and Forbes 2016).

Bacterial directed enzyme prodrug therapy (BDEPT) utilizes the tumor-specific localization of designed bacteria (carrying a gene coding for a prodrug conversion). This method harbors the enzyme based activation of systemically administered "prodrugs" distributed within the tumor for selective tumor destruction. This strategy can minimize the collateral damage that occurs during conventional therapeutic methods (Lehouritis et al. 2013). The hypoxic environment surrounding the tumors has been found as a probable therapeutic target (Harris 2002). On the basis of this information, Sasaki et al. (2006) designed an enzyme prodrug-based therapeutic strategy which includes the systemic co-administration of 5-fluorocytosine (5-FC) and cytosine deaminase-secreting *Bifidobacterium longum* bacteria. The bacterial strain was found to be selectively distributed in the hypoxic area of tumor. Once it is established in the tumor mass, an anti-cancer chemotherapeutic drug 5-fluorouracil (5-FU) was produced by conversion of 5-FC through the catalyzation by cytosine deaminase produced by the *B. longum*. This enzyme based application of probiotic strain led to targeted tumor suppression (Sasaki et al. 2006). This strategy may not only reduce the toxicity of chemotherapy drugs but also sensitize them to irradiation method.

14.5 Designer Probiotics in Inflammatory and Autoimmune Disease

Genetically designed lactic acid bacteria (LAB) have been engineered to express therapeutic heterologous proteins in the mucosa of the host. LABs can be used for delivery of the vaccine in order to provide protection against toxins and infectious diseases (Panteli and Forbes 2016). Also, they can be employed for delivering the immunotherapeutics to treat inflammatory diseases (Daniel et al. 2011). The microbiome has an influence on most aspects of human health and a non-healthy balance of microbiome can badly affect immune function, energy as well as oxidative stress. The significance of microbial diversity has been found in various studies, with a reduced diversity related with gastrointestinal tract (GIT) conditions like Crohn's disease (Demaison and Moreau 2002). Antibiotics have been used against such diseases, however, they can cause gut dysbiosis, resulting into dire adventitious infections as well as emergence of antibiotic-resistant bacteria. In this regard, the inactivation of antibiotics in the GIT represents an approach to protect colonic microbiota integrity and decrease antibiotic resistance. Beta-lactamase has been found to protect the gut microbiome against the damage via antibiotic inactivation in the GIT and offers a pharmacologic stewardship strategy to fight the emergence of antibiotic resistance (Connelly et al. 2019; Connelly et al. 2020).

Inflammatory bowel disease (IBDs) refers chronic inflammatory disorders of the intestines consisting of Ulcerative Colitis and Crohn's disease. Previously, researchers found that interleukin-10 (IL-10), anti-inflammatory cytokine, exhibit a crucial role in downregulating the inflammation associated with IBDs (Daniel et al. 2011). *L. lactis* was engineered to express IL-10 to prevent and treat colitis for the first time in year 2000. This approach resulted in 50% decrease in the colitis levels of mouse (Steidler et al. 2000). In the early 2000s, Vandenbroucke and his colleagues manipulated food-grade bacteria *L. lactis* for producing bioactive murine Trefoil factors (TFF); involved in reconstitution of the GIT. Oral administration of this modified bacteria enhanced expression of prostaglandin-endoperoxide synthase 2 and ameliorated Dextran sulfate sodium (DSS)-induced Colitis among IL-10-/- mice (Vandenbroucke et al. 2004). Later in 2006, *L. lactis* bacterium was genetically programmed to secret IL-10 against Crohn's disease. In the same year, phase 1 clinical trial with transgenic bacterium reported a decrease in disease activity (Braat et al. 2006). *Bacteriodes ovatus*, a commensal gut bacterium, was modified for delivering human keratinocyte growth factor-2 (KGF-2) in response to hemicellulose xylan and was able to limit the development of inflammation in the intestine thereby act as a therapeutic material for acute colitis (Hamady 2013). Further down the line, in 2014, *L. lactis*-mediated IL-10 cDNA delivery in host cells was found to confer protective influences against inflammation (Del Carmen et al. 2014b; Shigemori and Shimosato 2017; Singh et al. 2017). Overall, the use of engineered bacteria avoids systemic side effects provide a novel approach for the maintenance treatment for chronic intestinal disease. In 2015, a patent using engineered probiotic bacteria *L. lactis* and *Lactobacillus casei* expressing Elafin (trappin-2 protein) anti-inflammatory molecule was filed in order to help in prevention and treatment of IBD and irritable bowel syndrome (IBS). In the same year, *L. lactis* NZ9000 (NZ-HO) engineered to produce the anti-inflammatory recombinant mouse heme oxygenase-1 (rmHO-1) acting as a therapeutic agent against IBD by diminishing the expression levels of IL-1α and IL-6 (pro-inflammatory cytokines) in mouse colon was reported (Shigemori et al. 2015).

B. longum has been engineered to produce alpha-melanocyte-stimulating hormone, an anti-inflammatory peptide, against a-MSH ulcerative colitis model in vivo. Recombinant *B. longum* colonized in the intestinal gut of the rat, produced bioactive α-MSH, and exhibited significant anti-inflammatory consequence (Wei et al. 2016).

Further studies have demonstrated that *L. lactis* bacteria can be engineered to express thymic stromal lymphopoietin (TSLP) (Del Carmen et al. 2014b), IL-27 (Hanson et al. 2014), and heme oxygenase-1 (HO-1) (Sidel'nikov et al. 1972) for regulating acute inflammation of the GIT. In a study, arthritic mice were fed with milk fermented with recombinant *L. lactis* overexpressing colonization factor antigen I CFA/I fimbriae. As a result, the arthritis considerably improved via CD39+ Tregs producing IL-10 and TGF-beta, which in turn potently decreased TNF-a production as well as neutrophil influx into the joints (Maddaloni et al. 2015).

14.6 Designer Probiotics in Infections

With the advent of antibiotic consuming era, its inappropriate consumption along with overuse has driven the brisk emergence and rise of multidrug-resistant (or antimicrobial-resistant) pathogens. Lack of antibiotic options has led to the search for alternative means to control the present antimicrobial resistance crisis (Medina and Pieper 2016). One of the alternative methods involves the use of novel synthetic biology-driven antimicrobial strategy such as designer probiotics, owing to their controlled release of antimicrobial agents, enhanced specificity, and reduced danger pertaining to resistance against antibiotics. There are several kinds of Streptococcal infections that vary in severity from mild throat infections to pneumonia. Engineered probiotic strain of *Lactobacillus gasseri* NM713 designed to express streptococcal M6 protein (CRR6), have been found to protect the mice against streptococcal group A-mediated pharyngeal infections (Mansour and Abdelaziz 2016).

Recombinant *E. coli* carrying our synthetic genetic system for sensing and destroying infectious pathogen, e.g. planktonic *Pseudomonas aeruginosa* was first described in 2011 (Saeidi et al. 2011). Later, engineered *E. coli* capable of secreting the nuclease DNaseI and the antimicrobial peptide microcin S were employed for degradation of pathogenic *P. aeruginosa* with improved specificity, directed motility and efficiency. Recombinant *L. lactis* was engineered with genetic circuits to detect *Enterococcus faecalis's* sex pheromone and in response, express bacteriocins that possess antimicrobial potential against this multidrug-resistant bacteria (Borrero et al. 2015). Genetically engineered probiotics expressing the necessary antigens show potential as a promising vaccine candidate against infectious diseases (Bridge et al. 2016; Zadravec et al. 2016; Vishwakarma et al. 2015).

Designer probiotics have applications as anti-infective agents against life-threatening viral infections. *L. casei* has been engineered to produce a single-chain fragment variable (scFv) antibody which is capable of inhibiting cell-associated HIV-1 spread engineered lactobacilli producing HIV-1 fusion-inhibitor peptide with potent neutralizing capability have been assessed as potential bio-shields (Pusch et al. 2006). LAB designed to express single-domain antibodies (dAbs) protect the host against sexually transmitted viral infections such as HIV (Marcobal et al. 2016). Similarly, antiviral peptides expressing recombinant *Lactobacillus jensenii* reduced viral transmission and paved way for its application as topical live microbiocide (Lagenaur et al. 2011). An exopolysaccharide negative mutant of *Lactobacillus rhamnosus* GG engineered for surface display of VHH antibody fragment against rotavirus prevented against rotavirus-induced diarrhea (Álvarez et al. 2015). Designed bacteria expressing HIV Gag specific antigen elicited a strong immune response and thus exhibited implications as bacterial-based vaccine (Chamcha et al. 2015). Few genetically engineered probiotics are in human clinical trials. LACTIN-V; a recombinant probiotic for the treatment of bacterial vaginosis has recently been recorded for a phase II clinical trial. Designer probiotic *E. coli* Nissle 1917 elicited therapeutic actions against *P. aeruginosa*-mediated gut infection in mice and *Caenorhabditis elegans* models (Hwang et al. 2017). Oral

administration of *L. lactis* bacterium decreased intestinal *V. cholerae* burden and enhanced survival of infected mice through lactic acid production (Mao et al. 2018).

14.7 Designer Probiotics in Metabolic Diseases Management

Human metagenomics-wide association study has demonstrated the association of metabolic disorders such as Type 2 diabetes and obesity with dysbiosis, altered bacterial genes and their respective metabolic functions (Tilg and Moschen 2014). Current rise in the metabolic diseases such as cardiovascular disease (CVD), diabetes, obesity, etc. has paved way for the use of designer probiotics expressing therapeutic biomolecules or phage therapy. Engineered probiotics as dietary supplements or topical agents promote normal physiology along with high immunity to prevent the host organism against oxidative stress, infective agents, inflammatory disorders as well as autoimmune responses. Therapeutic paradigms like 2nd generation personalized designer probiotics may offer safe alternatives to conventional clinical interventions against chronic metabolic disorders (Peterson et al. 2015; Paton et al. 2012).

Formulations comprising of designer probiotics expressing anti-inflammatory cytokines and human proinsulin can be used against diabetes. Type 1 diabetes (T1D) is an incurable autoimmune disorder characterized by deficient tolerance to self-antigens of insulin producing pancreatic B cells (Piñero-Lambea et al. 2015). Non-obese mice orally administered with programmed *L. lactis* bacteria expressing HSP65 with tandem repeats of P277, led to reduction in the incidence of T1D in non-obese diabetic (NOD) mice (Chamcha et al. 2015). In another study against Type 1 diabetes mellitus (T1DM), oral intake of engineered *L. lactis* strains expressing recombinant HSP65-6IA2P2 protein in NOD mice reduced insulitis, prevented hyperglycemia, and ameliorated glucose tolerance and regulatory immune reactions (K. F. Liu et al. 2016). Delivery of IL-10 and T1D auto-antigen, i.e. glutamic acid decarboxylase (GAD65) via designed *L. lactis* prevented diabetes development in NOD mice (Robert et al. 2014).

Glucagon-like peptide 1 (GLP-1) is a peptide hormone secreted by intestinal L cells due to nutrient ingestion. It augments pancreatic function and exhibits a potential role in type 2 diabetes treatment. Recombinant human commensal bacteria *L. lactis* secreting GLP-1 when orally delivered to diabetic rats resulted in increase in insulin levels and up to 30% reduced levels of blood glucose thereby supporting the notion of recombinant commensal bacterial signaling to regulate enteric cellular functions in vivo (Duan et al. 2015). Likewise, oral administration of penetratin-fused GLP-1 by *B. longum* increased absorption of GLP-1 in the colon (Wei et al. 2015). Oral administration of designer probiotic bacterium *Lactobacillus paracasei* secreting Ang-(1-7) enhanced glucose tolerance by increasing insulin level, and decreased diabetes-induced harmful effects in retina and kidney (Duan et al. 2015).

High calorie intake and low energy expenditure, results in obesity thereby enhancing the risk of other life-threatening diseases. Currently existing medical

treatments and lifestyle amendments have provided incomplete protection against this malady. Prior research on human gut microbiota interventions along with transfer of disease-associated microbiota into mice has concluded the significance of a normal/healthy gut microbiota in host metabolism (Khan et al. 2014). Thus, it highlights the importance of microbiome-based therapeutics for treatment of metabolic diseases such as obesity. Incorporation of modified *E. coli* Nissle 1917 expressing N-acylphosphatidylethanolamines into the gut microbiota exhibits high potential to be used as an effective approach to deter the obesity in mice fed with high fat diet (Khan et al. 2014). In vivo administration of recombinant bacteria *E. coli* Nissle 1917 producing redox factor pyrroloquinoline quinone (pqq) along with fructose dehydrogenase, or with mannitol-2-dehydrogenase and glucose facilitator protein provided a beneficial role in reducing the severity of fructose induced hepatic steatosis (Somabhai et al. 2016).

Hypertension also called as high blood pressure is a leading cause of CVD. Recombinant probiotics expressing anti-hypertensive peptides have been used to curb this disease (Rao et al. 2009; Rao et al. 2011; Huang et al. 2012; Daliri et al. 2017). Urolithiasis is a metabolic kidney stone disease often occurred due to excessive intake of dietary oxalate-rich food. A recent study demonstrated that recombinant food-grade *Lactobacillus plantarum,* designed to produce oxalate decarboxylase (OxdC), degraded intestinal oxalate, reduced urea, calcium, and creatinine levels in vivo, thereby averted stone formation (Paul et al. 2018).

14.8 Designer Probiotics as Bio-Therapeutic Delivery Vehicles

Attenuated pathogens have been utilized for vaccine development, however, the main concern lies in their return to a virulent phenotype. The elevated expenses connected with the production of protein drug have enabled the design of less expensive and more robust probiotics to deliver engineered protein (Sleator 2015). Moreover, regular methods of protein drug delivery, like intramuscular and intravenous administration, are inherently invasive but essential due to less bioavailability upon oral administration. Oral administration and mucosal immune stimulation remains the advantages of using live vectors, such as LAB for biomaterial delivery (Sleator 2015; Chua et al. 2017; Sola-Oladokun et al. 2017; Ozdemir et al. 2018). A major benefit lies in the existence of the intestinal immune tolerance to various LAB leading to less danger of hypersensitivity after multiple administration (Wells and Mercenier 2008). Thus, ease of administration and lack of immune reaction makes LAB as a useful source for the delivery of biomaterials such as vaccines, antigens, and drugs inside the body of the host.

Our understanding of the mucosal immune system and gut flora has enhanced dramatically in recently years (Jiang et al. 2019). Exploring strategies of using designer bacteria as delivery vectors for vaccines for stimulating durable humoral and cellular immunity opens novel opportunities for their applications in healthcare industry (Jiang et al. 2019). In 2006, engineered probiotics-mediated protein drug delivery was examined through the oral administration of β-lactamase protein in rats.

Such delivery methodology resulted in two-to three fold rise in oral bioavailability as compared to control (Kaushal and Shao 2006). Later in 2009, a linear association of β-lactamase absorption with the *L. lactis* dose was determined, which underlined the potential of this method for sustained delivery of biological products (Kaushal and Shao 2009).

Designer probiotics-mediated vaccination against enterotoxigenic *E. coli* (Daniel et al. 2009), *Yersinia pseudotuberculosis* (Kaushal and Shao 2009), *Streptococcus pneumoniae* (De Lúcia Hernani et al. 2011), and *S. enterica* (Kajikawa et al. 2012) elicited specific and fast immune responses in vivo. Recombinant non-virulent *Vibrio cholerae* strain containing novel genetic circuit reprogrammed for surface expression of *Helicobacter pylori* adhesion antigen (HpaA) along with different colonization factor (CF) antigens of enterotoxigenic *E. coli* (ETEC), exhibited strong potential to be used as inactivated oral vaccine against *H. pylori* (Tobias et al. 2017).

Oral administration of antigens through engineered probiotic bacteria producing dendritic cell-targeting peptides combined with viral antigens is anticipated to provide protection from viral infections in the animals (Wang et al. 2017). Probiotic lactococci or lactobacilli being considered safe and naturally existing in humans and animals are chosen to express antigens of poultry and porcine viruses in order to express antibodies and cytokines for applications in oral vaccination. Designed *L. plantarum* producing spike protein originating from TGEV fused with DCpep increased $CD3^+$, $CD4^+$ T cells and MHC-II^+CD80^+ B cells, along with enhanced titers of serum IgG and secretory IgA in feces, thereby, validates the suitability of engineered probiotics via oral administration against the viral infections (Jin et al. 2018). *L. plantarum* was reprogrammed to produce influenza virus proteins. The chicks immunized with the antigen displayed characteristic humoral, mucosal and T cell-based immune response, thus enabling experimental chick to protect themselves against the viral infection (Yang et al. 2018a; Yang et al. 2018b).

In two studies, engineered probiotic *Lactobacillus acidophilus* and *L. gasseri* to delivered vaccines targeted against Bacillus anthracis. In both of them, anti-B. anthracis protective antigen (PA) antibody and T-cell-based immune responses were elicited. An expression system comprising of aggregation promoting actor (apf) gene facilitated the delivery of antibody fragments to GIT of murine model through *L. paracase* (Martín et al. 2011). Engineered lactococcal delivery strains have been designed to improve cellular invasion/host colonization and payload delivery. In a study conducted in 2009, a modified *L. lactis* strain designed to produce *Staphylococcus aureus* fibronectin-binding protein A (FbpA) effectively delivered DNA to human epithelial cells with comparable rates of internalization (Innocentin et al. 2009).

Bacterial spores have emerged as potential effective vaccine delivery vehicles. Lee et al reported that intranasal inoculation of *Bacillus subtilis* as a delivery vehicle for a vaccine against rota virus elicited protective immunity (Lee et al. 2010). *Clostridium difficile* is responsible for nosocomial infection and its two toxins, i.e. A and B are implicated as virulence factors. Similar to the aforementioned study, bacterial spore (*B. subtilis*) was employed as a delivery vector to assess the carboxy-terminal repeat domains of toxins A and toxin B as protective antigens. The

study demonstrated that mucosal immunization conferred protection against *C. difficile* (Permpoonpattana et al. 2011). Likewise in 2014, designer *B. subtilis* spores producing a *Mycobacterium tuberculosis* antigen, MPT64, was found to alleviate the bacterial burden in the mice lungs and stimulated antigen-specific secretion of Th1 cytokines after immunization (Sibley et al. 2014).

14.9 Designer Probiotics in Cognitive Health

A class of probiotics called "Psychobiotics," can produce and deliver neuroactive compounds to act on the gut–brain axis and deliver an antidepressant influence on the host organism (Dinan et al. 2013). Emerging body of evidence propose the notion of gut bacteria exhibiting an effect on the brain chemistry as well as behavior of the organism (Moloney et al. 2014). In vitro and in vivo model animal research envisages the role of gut microbes and their metabolites in affecting neural circuitry, central nervous system development, stress-related responses, and normal behaviors (Forsythe et al. 2012). In vivo data elucidates ingestion of a commensal bacterium *L. rhamnosus* JB-1 provides immunoregulatory effects, via impacting nerve-dependent colon migrating motor complexes, cognitive elements, behavioral features and enteric nerve actions (Al-Nedawi et al. 2015). LAB decrease concentration of neurotoxic substances like indoles and ammonia (Kopp-Hoolihan 2001). The gut bacteria exhibit an essential part in maintaining cortical myelination and might be considered as a therapeutic target in myelination-related ailments like multiple sclerosis (MS) (Brichacek et al. 2013).

Studies suggest the potential for microbial-based therapeutic strategies in mental health, as there appears to have association of gut microbiota with autism, depressive illness as well as human moods and behavior (Dash et al. 2015; Al-Nedawi et al. 2015). Although there have been few psychobiotic studies with human trials, however, additional mechanistic studies and more placebo-controlled trials are required to make necessary inferences regarding the effectiveness of probiotics in application associated with mental health (Forsythe et al. 2012). The aforementioned studies envision the future clinical applications of genetically engineered probiotics in the field of cognitive health. Further studies into the mechanism of psychobiotics would lead to the production of designer agents having applications in biotherapeutics for curbing psychological ailments in near future.

14.10 Genetic Engineering-Mediated Engineering of Designer Probiotics

Programmable nuclease such as CRISPR-mediated genome engineering technique has modified the landscape of genome editing in food and pharmaceutical industries, and enlightened the route for next-generation prokaryotic engineering for futuristic therapeutic and prophylactic applications. Amalgamation of CRISPR-facilitated editing with the field of synthetic biology predicts site-specific delivery and rational

programmable biological containment design. CRISPR-driven editing toolkits for prokaryotic engineering have been engineered for *E. coli*, *B. subtilis*, Clostridium, Corynebacterium, and actinomycetes in order to facilitate, gene insertion, gene deletion and precise base editing for extensive applications (Mougiakos et al. 2016; Liu et al. 2017; Westbrook et al. 2016). Cas9-based single-stranded DNA recombineering was initially achieved in *Lactobacillus reuteri* of Lactobacillus species for small deletions (Oh and Van Pijkeren 2014). Later, SpyCas9-assisted gene loss and gain were executed in *L. casei* (Song et al. 2017); and the point mutations in *L. plantarum* (Leenay et al. 2019). CRISPR technique dependent on the varied repertoire of endogenous CRISPR-Cas systems in lactobacilli bacterium would promote designer probiotics bioengineering via developing health functionalities using probiotics as chassis of delivery as well modifying the inherent functions of the probiotic bacteria to provide stability.

In few embodiments, Zinc Finger Nuclease (ZFNs) or Transcription Activator-Like Effector Nuclease (TALENs) might be considered a viable substitution for CRISPR/Cas nucleases in a designer probiotic, provided the ZFN or TALEN is engineered to target a particular DNA sequence for degradation. ZFNs and TALENs comprise of modular protein domains, where each domain exhibits the specific binding capacity to a particular DNA base pair. ZFNs' individual modular domains target a variety of 3 base pair sequences. Engineered ZFNs are fusion proteins, comprising of three ZFN modules that target a particular 9bp sequence. ZFNs-mediated gene editing is a potential treatment strategy against diseases such as Duchenne muscular dystrophy (Ousterout et al. 2015), Hemophilia (Anguela et al. 2013), and HIV (Tebas et al. 2014). Similarly, engineered TALENs consisting of a fusion protein of modular TALEN domains can be designed in order to target a particular bp sequences. TALENs-mediated gene editing strategy has shown contribution against Hepatitis B Virus (HBV) infraction (Bloom et al. 2013) and Duchenne muscular dystrophy (Li et al. 2015).

14.11 Designer Probiotics as Nutraceuticals

Nutraceuticals such as omega-3 long chain polyunsaturated fatty acids (LC-PUFA) are acknowledged owing to their actions on human nutrition, neurodevelopment status, and prevention of CVD (Voigt et al. 2002; Das 2003; Demaison and Moreau 2002; DeFilippis et al. 2010; Gong et al. 2014). Due to insufficient supply of LC-PUFA through its natural sources, i.e. marine, the metabolic engineering of oleaginous micro-organisms for bulk supply of this nutraceutical could be a likely solution. Likewise, there is a massive increase in the demand of antioxidants owing to their beneficial effects. Progress in metabolic engineering efforts, for the effective reconstitution of heterologous pathways in suitable microbe is essential for generation of antioxidant food ingredients (Lin et al. 2014).

Probiotics have the ability to survive in the stressful environmental conditions of the GIT owing to their tolerance of gastrointestinal stresses. Well-regulated genetic system allows for immediate response and resistance against such stresses. In this

regard, initially stress specific genes ease the immediate stress via degradation of toxic compounds or maintenance of pH. It is then followed by the universal stress response system of repairing the DNA and proteins. MutS has a crucial role in repairing the bacterial DNA damage and ensure bacterial survival in the GIT (Li et al. 2015). MutS is a critical enzyme that exhibits a crucial role in recombinational events as well as correcting mismatched bases during DNA replication and other biological developments (Morita et al. 2010). MutS enzyme recognizes the base mismatches during replication and stimulates the downstream processes essential in the process of DNA mismatch repair (MMR) (Modrich 2006; Fukui 2010). The small MutS-related (Smr) endonuclease domain has been discovered in the C-terminal domain of MutS2 anti-recombination protein (Kang et al. 2005). MutS2 suppresses homologous recombination via endonucleolytic resolution of early intermediates in the process. This endonuclease property of MutS2 is derived from its Smr domain (Fukui and Kuramitsu 2011). The basic processes and proteins involved in the early MMR reactions are conserved in almost all organisms ranging from archae, bacteria to eukaryotes such as humans (Fukui 2010). Till now, two kinds of MMRs have been known: *E. coli* type and the human type (Fukui 2010). It can be assumed that the probiotics overexpressing MutS2 would be able to easily survive the harsh stressful environment of GIT.

14.12 Designer Probiotics with Antimicrobial Peptides

The focus of modern medicine is to design novel therapies to combat quickly growing microbial resistance against antibiotics. In this regard, the probiotics and Antimicrobial peptides (AMPs) serve as alternate approaches to deal with drug-resistant pathogens for providing health benefits. Effective drugs for targeting biological problems can be developed by using cationic AMPs that primarily function through membrane-active systems (Ong et al. 2014). Owing to few hurdles encompassing the production, purification and delivery of these peptides, new mathematical simulations models, and computational strategies are required. Such novel strategies will allow researchers to identify, evaluate, and predict AMPs and their respective activity from genomic databases (Andreu and Torrent 2014; Amaral et al. 2012). Another approach includes employing a combinatorial strategy of AMP producing probiotic. In this regard, high-titer of recombinant AMPs could be produced from designer bacteria via molecular cloning of AMP genes. Such novel approach fetches the combined advantages of probiotics as AMPs simultaneously.

Transcriptome and high-throughput sequencing analyses show that non-coding RNAs (ncRNAs) can be found in several organisms (Kapusta and Feschotte 2014). ncRNAs have been implicated various l processes such as plant growth, development and its responses to environmental stresses (Mercer et al. 2009) (Kapusta and Feschotte 2014). ncRNA includes long non-coding RNAs (lncRNAs) and microRNAs (miRNAs). Though bacteria harbor fewer long ncRNAs as compared to the eukaryotes, the known ncRNAs in bacteria perform vital functions in the processes of metabolism, information transfer, and physiological adaptation (Cech

and Steitz 2014). Structured bacterial ncRNAs which have more than 350 nucleotides are catalytic RNAs or they function in catalytic complexes. ncRNAs having sophisticated structures are usually ribozymes (Harris and Breaker 2018). ncRNA is present in various bacterial strains such as *Lactobacillus salivarius* (Cousin et al. 2017).

ncRNAs, such as lncRNAs and miRNAs, were initially anticipated to be incapable for proteins/peptide expression (Mercer et al. 2009; Rogers and Chen 2013; Patil et al. 2014). Increasing body of evidence indicate that lncRNAs can encode small peptides (Ruiz-Orera et al. 2014). In mammals the lncRNA-encoded micropeptides possess either stimulate or inhibit their target genes (Anderson et al. 2015; Nelson et al. 2016). In mammals, myoregulin (MLN), a lncRNA-encoded micropeptide, has been found to have a role in regulating muscle performance (Anderson et al. 2015). Another lncRNA-encoded peptide, called dwarf open reading frame (DWORF), increases sarcoendoplasmic reticulum calcium transport ATPase (SERCA) activity and Ca^{2+} load through displacing the inhibitors of SERCA and decreasing their inhibitory function (Nelson et al. 2016). Approximately 30,000 ncRNAs have been discovered in plants with more than 1700 transcripts designated as ncRNAs are present in Arabidopsis alone (Liu et al. 2015). The functionally characterized plant lncRNA-encoded peptides include: Induced by phosphate starvation1 (IPS1) (required for phosphate uptake), Early nodulin 40 (ENOD40) (needed for the symbiotic interaction between bacteria and plant), Long-day-specific male-fertility-associated RNA (LDMAR) (involved in controlling photoperiod-sensitive male sterility) and COLDAIR & COOLAIR (affecting flowering time by through the transcription of Flowering Locus C) (Lv et al. 2016).

MicroRNAs (miRNAs) derived from the primary miRNAs (pri-miRNAs) are short RNA molecules generally around 21 nucleotides in length and negatively regulate gene expression levels by binding to and cleaving the mRNAs or otherwise inhibiting their translation into proteins (Rogers and Chen 2013). Pri-miRNAs harbors short open reading frames (ORFs) which encode regulatory peptides, called miRNA-encoded peptides (miPEPs), indicating that pri-miRNAs exhibit protein-coding as well as non-coding functions (Nelson et al. 2016). The discovery of miPEPs is in line with reports that a huge amount of micropeptides are encoded by previously unannotated short ORFs in lncRNAs (Ruiz-Orera et al. 2014). The local miPEPs' expression is detected through specific antibodies, and that the patterns of expression are same as the corresponding miRNAs. miRNAs are generally considered as non-coding RNAs, however, their primary transcripts encode small functional peptides, the miPEPs, that specifically activate their miRNA transcription in a positive loop and ultimately suppress the target genes. Examples of two such miPEPs include miPEP165a from Arabidopsis and miPEP171b from *Medicago truncatula*. Their exogenous application or overexpression increases the expression levels of their corresponding miRNAs, thereby sequentially potentiating the downregulation of the target genes associated with root development (Mohammad et al. 2019). Plants like legumes (beans, lentil, and peas) contain beneficial bacteria in the nodules attached to their roots. These beneficial bacteria "fix" vital nitrogen and turn it into a form that can be easily utilized by the plant. In this regard, the

treatment of exogenous miPEP172c to enhance the nodulation between symbiotic bacteria and soybean legume plant is of high significance (Anderson et al. 2015). Due to miPEP-mediated enhanced root nodule formation containing these nitrogen fixing bacteria, the farmers could use less quantity of chemical fertilizers to overcome the nitrogen required by the plant. Owing to the role of miPEPs in the agriculture, one can presume a future in which they could be expressed by bacterium to enhance healthy plant production.

14.13 Aptamer-Based Applications

L. acidophilus is an important probiotic strain that has applications in immune system stimulation and improvement of digestion. Monitoring of probiotic bacterial strains is critical for high purity, quality, and safety control due to its large applications. Aptamer-decorated porous silicon biosensors have been found to be a reliable procedure for the specific detection of *L. acidophilus* at concentrations relevant for probiotic products (Lv et al. 2016). Similarly, in other study aptamer CCFM641-5 was employed to detect *Bifidobacterium bifidum* probiotic bacteria, which is often used against IBD (Hu et al. 2017). Aptamer-based capture probes have been produced against various bacteria such as *S. aureus* (Rhizobium 2013; Turek et al. 2013), *M. tuberculosis* (Chen et al. 2007), Campylobacter jejuni (Dwivedi et al. 2010), *L. casei* (Song et al. 2019), *E. coli* (So et al. 2008) and *S. enterica* (Singh et al. 2012).

14.14 Designer Probiotics and the Livestock Industry

Currently, the livestock industry is facing the dilemma of the scarcity of quality fodder impeding the livestock production in the underdeveloped countries. Humans and animals (especially high yielding livestock) require readily fermentable carbohydrate as well as protein-rich food sources in order to perform their activities. A high population rate has led to rise in the demand for the production animals (for meat) and pulse crops. At the current moment the conventional silages and the metabolic ability of rumen microbes is inadequate to meet the demand of nutrients requirement for the livestock. Keeping in mind the present scenario, the feed additives such as recombinant probiotics can be utilized. The need of the hour is to design genetically engineered micro-organisms capable of colonizing the GIT in order to enhance nutrients supply to the food consumers. In this way, the nutrient requirement can be achieved from low-quality forages, rather than high quality and expensive protein concentrates (Singh et al. 2019). Clinical mastitis can present in a wide degree of severity that can vary from mild to moderate to severe. The degree of infection and the symptoms present may depend on several factors, such as the cow's nutritional or immune status, responsible for the inflammation (Isolauri 2001). Changes in the composition of milk even in cows with subclinical mastitis can lead to major changes in the composition of the milk proteins. While the overall

protein content may not be affected, variations in protein types can be affected by leaching (low-quality) blood serum proteins into milk, casein, an essential protein contained in healthy milk, may be greatly decreased in sub-mastic cows, and another problem is that casein is closely related to milk output calcium levels (Maxson and Mitchell 2016).

14.15 Probiotics in Poultry Nutrition

In various nations, the poultry sector has become a major financial activity. Poultry is subjected to demanding circumstances in large scale rearing facilities, disease-related problems and environment degradation resulting in significant financial losses. Poultry is the cheapest source of animal protein and contributes to the rising demand for animal food worldwide (Marcobal et al. 2016). Spending and export in poultry products is increasing, making it the second largest source of meat after pork (Borrero et al. 2015).

Probiotics can enhance the development rate of broiler chicken (Afsharmanesh and Sadaghi 2014) and control or prevent enteric illnesses, including salmonellosis (Bogucka et al. 2019), necrotic enteritis (Jayaraman et al. 2013), and coccidiosis (Dalloul et al. n.d.).

Probiotics mode of action in poultry includes: (a) preserving normal intestinal microflora by competitive exclusion and antagonism (Nurmi and Rantala, 1973), (b) enhancing digestive enzyme activity by altering metabolism and reducing bacterial enzyme activity (Jin et al. 2000), (c) enhancing feed consumption and digestion (Awad et al. 2006), and (d) stimulating the immune system (Koenen et al. 2004).

Probiotics increased broiler growth rates better than antibiotic growth promoters (avilamycin) (Zhang and Kim 2014) and other antibiotic growth promoters replacements such as phytochemicals (e.g. essential oils) (Takahashi et al. 2019). The overall applicability of the probiotic strategy as an alternative to antibiotic growth promoters, however, is still not well known.

Probiotics ranging from non-spore forming LAB to spore formers and yeast were evaluated for their ability to increase to improve rates of growth of commercial poultry production (Fang et al. 2018; Afsharmanesh and Sadaghi 2014). The increase in the development rate in probiotic treated birds has in many instances been correlated with enhanced feed consumption and enhanced feed effectiveness relative to untreated birds (Zhang and Kim 2014; Caluwaerts et al. 2010). One of the interesting findings made in probiotic poultry feed studies show that some encourage development at the beginning (early) stage (Bai et al. 2013) and other in the cultivator (later) stage (Palamidi et al. 2017).

The probiotic bacteria must satisfy the following requirements, they must be a stable inhabitant of the intestine, and they must be able to adhere to the intestinal epithelium to conquer possible obstacles, such as low stomach pH, bile acids in the intestines, and the competition in the GIT against other micro-organisms. Several in vitro assays were produced for the pre-selection of probiotics strains. (Ehrmann et al. 2002). The competitiveness of the most promising strains selected through

in vitro assays was evaluated in vivo (Garriga et al. 1998). Therefore, potential probiotics can also exert their beneficial effects (e.g., enhanced nutrition and increased immune response) in the host. Eventually, the probiotic must be viable and physically suitable for industrial processes (e.g., lyophilized) under normal storage conditions.

14.16 Designer Probiotics in Egg Nutrition

Studies show improved egg production with dietary supplementation from probiotics (Kurtoglu et al. 2004), which has no impact on egg production (Mikulski et al. 2012). However, probiotics have variable effects on the quality of feed usage in the laying hens. Cholesterol reduction in egg yolk is one of the most beneficial effects probiotics have on the consistency of the eggs. LAB (Park et al. 2016), Bacillus spores (Kurtoglu et al. 2004), and yeast have lowered yolk cholesterol (Aliakbarpour et al. 2012).

14.17 Designer Probiotics in Pig Nutrition

Although banned in some countries, including the EU, there is still widespread sub-therapeutic use of antibiotics in feed in the swine industry to prevent diarrhea and improve efficiency. This replacement has been more extensively studied for monogastrics in poultry than in pigs. Generalizations are complicated, as with other animals, due to differences in the microorganism used, does and duration of treatment husbandry practices (Liao and Nyachoti 2017). Several probiotics were used to increase the pigs' performance. Commercial BioPlus 2B probiotic drug, containing B. subtili*s* and B. licheniformis, was a viable nondrop replacement of AGPs (neomycin, oxytetracycline, tylosin) and with no increase in the cost of production (Kritas and Morrison 2005). BioPlus 2B also increased weight gain by up to 8% and dose-dependent feeding efficiency by up to 10% in growing and finisher pigs.

14.18 Designer Probiotics in Ruminant Nutrition

The rumen has a complex microbial ecology, where rumen micro-organisms degrade host-ingested polysaccharides and protein, resulting in the synthesis of SCFAs and microbial protein, which the host uses as the source of energy and nutrition. There is growing international interest in manipulating the rumen environment to increase the efficiency of the ruminal fermentation processes to increase animal productivity and reduce undesirable by-products, such as methane. Yeast (*S. cerevisiae*) is a widely used probiotic in ruminants causing rumen and nutrient degradation in the microbial population dynamics. Lactic acid-producing bacteria are another important group of probiotics. In dairy animals, probiotics can increase the milk yield. *E. faecium* and *S. cerevisiae* increased average milk yield by 2.3 liters per cow per day. Probiotic

containing a mixture of micro-organisms (*L. reuteri* DDL 19, *L. Alimentarius* DDL 48, *E. faecium* DDE 39 and *Bi. bifidum* DDBA) resulted in an average body weight gain by 9% when fed to goats for eight weeks (Marco et al. 2010).

14.19 Designer Probiotics in Aquaculture

Several bacterial species are attenuated in the field of aquaculture to exploit multivalent vaccines which effectively protect aquatic animals from infections of multiple aquatic pathogenic bacteria. For example, attenuated *Vibrio anguillarum* MVAV6203 was designed to induce dual protective immune response *Aeromonas hydrophila's* foreign antigen GAPDH, and the live-bacteria itself (Zhao et al. 2011). However, *Vibrio anguillarum* and *Edwardsiella tarda* are the two major aquatic pathogens that cause vibriosis anguillarum and edwardsiella tarda in various aquaculture fish species (Gao et al. 2014).

In aquaculture, various parameters are to be considered when choosing suitable probiotic strain, host origin, safety of strain, production of antimicrobial substances, host immune response. Additionally, three potential probiotic strains have demonstrated the ability to suppress *Lactococcus garvieae* under in vitro conditions. *L. lactis, Leuconostoc mesenteroides* and *Lactococcus plantarum* (Marco et al. 2010). Since unwanted micro-organisms can cause unwanted effects on the host, selection of probiotics is very important. Probiotics are more likely to interact with resident micro-organisms (Hoseinifar et al. 2018). Certainly another essential property of a probiotic is its capacity to colonize the intestinal tract or other epithelial surface (Gueimonde et al. 2013). Finally, *L. lactis*, *Lactobacillus sakei,* and *Leuc. Mesenteroides*, isolated from salmonid microbiota, were administered to brown trout (Salmo trutta) and found that these strains can live in intestinal mucus membrane (Balcázar et al. 2007).

The first studies suggesting that bacteria can regulate fish disease and enable nutrient regeneration as well as serving as food (Mikulski et al. 2012). The species Lactobacillus, Carnobacterium species, and Bacillus are the Gram-positive bacteria most commonly known in aquaculture as probiotics (Balcázar et al. 2007). LAB do not produce spores, and are non-motile and create lactic acid as their main end product during carbohydrate fermentation. Lactobacillus and Carnobacterium probiotics are active against edwardsiellosis, furunculosis, and vibriosis (Hoseinifar et al. 2018). However, a study shows a large increase in the mean weight and survival rate of turbot larvae fed with rotifers enriched with Lactobacillus and Carnobacterium and these strains provided substantial defense against Lactobacillus and Carnobacterium (Hoseinifar et al. 2018). Rod-shaped, Gram-positive, rod-shaped bacteria form bacteria constitutes genus Bacillus which is characterized by their ability to produce endospores in presence of unfavorable environmental conditions. Most species of Bacillus do no harm to humans or animals, and are essential secondary metabolite sources, including antibiotics and enzymes (Hoseinifar et al. 2018). *Bacillus* species improve aquaculture water quality (Monteagudo-Mera et al. 2019). However, denitrification characteristics of Bacillus

sp. Strain YX-6 were evaluated which showed that this strain could degrade concentrations of nitrite nitrogen (nitrite-N) concentrations under aerobic conditions (Varankovich et al. 2015).

Gram-negative probiotics used in aquaculture include Aeromonas, Enterobacter, Pseudomonas, Shewanella, and Vibrio species (Nayak 2010). Additionally, use of Gram-negative bacteria is accompanied by risk of transfer of encoding resistance to virulence from genetic material (Rocha-Ramírez et al. 2017). A substantially improved survival rate has been demonstrated in *Pecten maximus* treated with *Alteromonas haloplanktis* following challenge with *V. anguillarum* (Kritas and Morrison 2005). *Pseudomonas fluorescens* decreased mortality of rainbow trout infected with a *V. anguillarum* strain (Farzanfar 2006). Recent studies have observed the effect probiotic bacteria belonging to genera with pathogenic organisms, such as Pseudomonas, Vibrio or Aeromonas, while regulatory agencies are responsible for their use in aquaculture. (Jin et al. 2000).

Yeasts have the benefit of not being with antibiotics and will help recover the usual microbiota antibiotic treatment. This may also be an effective species, as certain strains synthesize and secrete specific polyamine molecules (Caruffo et al. 2015) and have strong adherence to intestinal mucus fish. The probiotic properties of both bacteria and yeasts were measured, and it was found that both survival and body weight were increased (Varankovich et al. 2015).

14.20 Safety Concerns

An approach to address the aforementioned concerns of horizontal gene transfer is to employ biological containment methodology aimed at designing genetically modified microbes that can survive only under specific conditions. Active biocontainment includes the conditional production of a bacterial toxin/antitoxin via controlled gene expression regulated through environmental cues. Passive biocontainment approach involves bacterial growth/proliferation depending on the complementation of auxotrophy or gene defect, through supplementation of essential metabolite or another genetic element (Broaders et al. 2013). Genomic recoded *E. coli* strains were constructed by introducing expanded codons for non-standard amino acids (NSAAs) into essential genes. The resultant probiotic strains strictly relied on the availability of NSAAs for their growth. Another approach included the introduction of ligand-dependent essential genes into an *E. coli* BL-21 strain. Owing to this, strictly after adding the synthetic chemical benzothiazole in the culture medium, the bacterial growth was rescued. This strategy is safe as the absence of such synthetic chemicals in nature renders inhibitions to the bacterial growth in the open atmosphere (Turek et al. 2013). Similarly engineered organism's dependency on Phosphite (Pt) has also been used as a strategy for biocontainment (Hirota et al. 2017).

Applications of recombinant bacteria are utilized at industrial scale for the generation of metabolites (Van Huynegem et al. 2009; Van Huynegem et al. 2009; del Carmen et al. 2014a; Chen et al. 2014). However, at the same time, health and the

environmental applications of modified micro-organisms demand stringent security and safety procedures. The safety of probiotic administration is related with the intended application, which involves dose and duration of consumption, assessing the potential vulnerability of the consumer, the manner as well as the frequency of probiotic administration (Sanders et al. n.d.).

Gene expression can be regulated through creation of mutant promoter or ribosomal binding site (RBS) libraries or by means of an inducible system in which an exogenous molecule drives expression of the gene of interest. In this regard, the use of genome sequencing expertise to guarantee the absence of genes of interest is essential prior to use that candidate probiotic strains. Clinical studies with engineered LAB's must address the deliberate release recombinant bacterium into the environment proper guidelines must be ensured regarding the environmental containment as well as its eradication (Kota et al. 2018). The need of the hour is to have a vivid comprehension of probiotic interactions with the host as well as colonizing microbes to overcome the issue underlying negative probiotic effects (Kota et al. 2018).

Paramount concern has been associated with the safety, as these engineered probiotics consist of foreign genetic elements required to stimulate immunomodulatory activities, antigenicity and possess capability to influence metabolic functions of the host. Owing to this, culture independent metagenomic studies as well as large, well-designed, randomized controlled clinical trials have to be administered carefully. Expert healthcare professionals' consultation is vital before designer probiotic formulations are consumed, as single strain/species of engineered probiotics may require unique preparation and exhibit different effects from others species/strain. Medical concerns such as overstimulation of the immune system, elevated levels of harmful metabolic actions, acute inflammatory responses, and so on confer chief concerns of the use of designer bacteria supplements. Moreover, designer probiotic-mediated high overproduction of antagonistic elements might hinder the growth and production of other essential gut bacteria. Also, the transferable genes for the resistance to antibiotic often carry a theoretical chance of transfer to a less harmless gut microbe. It is noteworthy that the transfer of the drug resistance gene and its chances of survival are more common among lactobacilli, and from lactobacilli to pathogens and vice versa (Stadlbauer 2015). Therefore, the probiotic's genetic stability over time and its risk for pathogenicity should be tested prior to its application. (del Carmen et al. 2014a).

Safety is a crucial concern in the therapeutic use of microbes to guarantee exclusion or prevention of adverse circumstances under specific conditions. Probiotics are unique as they are alive when administered, and may possess the potential for infection or toxin production. Though probiotics are valued universally for their pro-health characteristics however, some side effects, including mutagenesis, overstimulation of immune system, damaging metabolic functions, etc. have been found. Some studies report gastrointestinal side effects and hyperstimulation of immune system (Rosander et al. 2008), overall, the studies are indecisive on the application and safety of probiotics (Stadlbauer 2015). Currently, few studies on lactobacilli pathogenesis, and the risk of its infection is increased (Awad et al. 2006).

14.21 Conclusions

Exploiting microbiota is emerging as an important futuristic substitute to prevent multidrug resistance faced by the use of antibiotics. As designer probiotics might restore microbiota as well as the health effectively with site-specificity, the challenges and chief obstructions linked with their applications must undergo in-depth investigation. The designer probiotics-mediated therapeutic interventions require a critical analysis prior to being recommended for human trials. Few instances delivery of IL-10 in Crohn's disease, (clinical trials, phase IIa), is the prime example of successful application of designer LAB in human trial, thereby paving way to be considered a serious realistic therapeutic solution option for human.

The development in the field of genetically engineered probiotics would lead to a cut in production, delivery as well as storage costs by circumventing the fragility and short half-life span related to conventional therapies. However, at present the consumers' opinion and acceptance of designer probiotics remain a difficult challenge. Along with the application of comprehensive risk-benefit analyses and the effective use of biological containment protocols, there is a dire need to provide balanced unbiased objective information and education to the consumers on this topic. These steps will overcome the challenges and allow designer probiotics technology to gain a wider acceptance in the general population in near future.

Another matter revolves around the concern that many designer probiotics have orally administration. Owing to this, it is necessary that the designer probiotics must be capable of surviving through technological and gastrointestinal stresses. It is critical that these designed strains should have scientifically validated health and technological properties as well as safety to be generated at a large scale. It is a challenge to keep designed strains viable in a huge amount so as to confer the advantages to the host. Till date, the effects of exogenous probiotics administration on commensal bacteria or the whole microbiota have not been widely studied. Therefore, it is necessary to address such aspects in further studies related to bioengineered probiotics. Thus, the concerns encompassing detailed comprehension of the issues associated with the composition of gut microbiome and consequences related to externally introduced designer probiotic on the pathogen(s) of interest must be the prioritized area of future research.

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A Glance to the Patent World of Probiotics 15

Vivek P. Chavda, Arumugam Meyyappan, Disha Chavda, and Moinuddin Soniwala

Abstract

Patents are important when it comes to probiotics business whether it is food or therapeutic supplements. Probiotics are getting attractive niche for the same. According to World Health Organization (WHO), Probiotics are viable microorganisms and when administered in sufficient numbers, confer health benefits to the host. These are mainly explored for the treatment of gastrointestinal diseases, enhancement of mineral bioavailability, antioxidant potential, and immunomodulation. More than 524 patents granted approval (in the USA and Europe) from more than 2500 patent applications filed. Genetic engineering and a boom in the biotechnology field have brought its extension towards the development of new patentable bacterial strains with added advantages over the natural strain. Recent patents have revealed that probiotic bacteria can effectively produce metallic nanoparticles and found applications in cosmetics, pharmaceutics, medicine, and biotechnology. This chapter attempt to highlight the global market potential of probiotics in various fields. A complete patent scenario of the probiotics with different medicinal field shall be explored to get the overall picture of the probiotic business potential.

V. P. Chavda (✉)
Formulation and Development, Dr Reddy's Laboratory (Biotech Division), Hyderabad, Telangana, India

A. Meyyappan
Formulation & Development, Lupin Research Park (Biotech Division), Pune, India

D. Chavda
Analytical Development, Otsuka Pharmaceutical Ltd, Ahmedabad, India

M. Soniwala
Department of Pharmaceutics, B.K. Modi Government Pharmacy College, Rajkot, India

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Keywords

Probiotics · Patents · Bacterial strains · Pre-biotic · Genetic engineering

15.1 Introduction

Large number of microorganisms are living both in and out of humans. This microbial population is estimated to over 100 trillion and known as the microbiome or human microbiome. Probiotics are live microorganisms intended to provide health benefits when consumed, generally by improving or restoring the gut flora (Dixit et al. 2016). These are having considerable amount of safety. The probiotic market has excelled in foods and supplement industry (Soccol et al. 2010). The joint Food and Agriculture Organization (FAO), World Health Organization (WHO), the International Life Science Institute (ILSI), and the European Food and Feed Cultures Association (EFFCA) have defined probiotics that these are viable microorganisms and when administered in sufficient numbers, confer health benefits to the host (Nagpal et al. 2012). Many microbiome-based inventions are likely to be viewed as "laws of nature" or "natural phenomena subject to a heightened level of scrutiny by patent reviewers and the courts, as the proposed inventions are eligible for patent protection. Patent statutes claimed that, "products of nature" are excluded from being patented (Yarbrough and Liu 2019). Product of nature means i.e. purely natural product, such as plant that occurs in nature, an unmodified extract of a plant and bacterium as it exists in nature would not be patentable subject matter (de Simone 2019). A bacterial strain developed by organization in the lab can potentially be patentable. Genetic engineering and a boom in the biotechnology field have brought its extension towards the development of new patentable bacterial strains with added advantages over the natural strain (Wasserman 2018).

Not only new strain, but combination of known species might be patentable if one proves its efficacy (Soccol et al. 2010). Inventions relating to probiotics such as a new manufacturing method, a new method of preserving the bacteria, or other such probiotic-related activities can also be patentable (Grabowski et al. 2017).

Regulation of probiotics varies between regions. Unless they make specific disease-related health claims, probiotics are regulated as food supplements and regulation is focused on the legitimacy of any claims, rather than efficacy, safety, and quality (Nagpal et al. 2012). Many properties of probiotics are strain-specific, and safety and efficacy findings associated with specific formulations should not be generalized to other probiotic products. Current trademark law and the lack of stringent regulation of probiotic manufacturing mean that the trademark owner can commercialize any formulation under the same brand, even if significantly different from the original (van Belkum and Nieuwenhuis 2007). These regulatory deficits may have serious consequences for patients where probiotics are used as part of clinical guideline-recommended management of serious conditions such as

inflammatory bowel diseases, and may make doctors liable for prescribing a formulation not previously tested for safety and efficacy (Sanders 1998a).

Protecting IP rights in probiotics and microbiome-focused technologies has unique features that provide a twist on standard pharmaceutical and biotechnology patent practice. In recent years, the pharmaceutical industry has experienced a wave of patent challenges (van Belkum and Nieuwenhuis 2007). This topic remains an important issue for further research, particularly different public policies governing patent challenges for biosimilar and biologics compared with those for generic drugs and new chemical entities. The number of patent challenges has increased rapidly since the late 1990s. Many countries do not have the equivalent to the US's Orange book, and as a result health officials are wasting time establishing whether drug patents have been taken out on medicines (Kort and Sybesma 2012). However, whether company is pursuing patent protection of their own or not, it is important to be aware of the other side i.e. patent infringement. Patents can not only protect a company's innovations by creating a defensive weave around its creation but can also allow a company to consume its competitor's business aggressively. Companies try to build a strong and comprehensive portfolio of patents to make it difficult for others as the rivals always run a risk of (knowingly or unknowingly) trespassing or infringing of claims (Grabowski et al. 2017; Kort and Sybesma 2012). Hence, patents as one of the forms of IPR have become crucial strategic business tools of competitive intelligence in today's commercial scenario. It can boost a company's commercial prospects and in turn, increase its brand value. The next few years will be telling us the desired outcome for the microbiome from both a business and patent perspective (Soccol et al. 2010).

15.2 Commercial Probiotic Strain and Its Selection Criteria

According to the suggestions of the WHO, FAO, and EFSA (the European Food Safety Authority), in the Probiotic strains selection process safety and functionality criteria have to be on top goal (Fig. 15.1) (McVeigh et al. 2006). Probiotic characteristics are basically associated with the few selected bacterial strains with adequate safety margin (Table 15.1) (Hill et al. 2014). When one defines the safety criteria of a strain selection, it is basically related with its origin, the absence of association with pathogenic cultures, and the antibiotic resistance profile. On the other side of the coin i.e. functional aspects is related with its ability to sustain in the GI tract and its ability to influence immune function. Apart from safety and functionality requirements, stability of probiotic strains has gained momentum nowadays i.e. their ability to survive and maintain their quality throughout the storage and distribution processes (Lee 2009).

Establishment of probiotics dosage regimen, type of carrier/matrix, and their combination products are the area which requires key attention from the scientific community for future endeavor. It is always advisable to check the new developed strain with that of available strain and check the betterment aspect in terms of quality, safety, and efficacy.

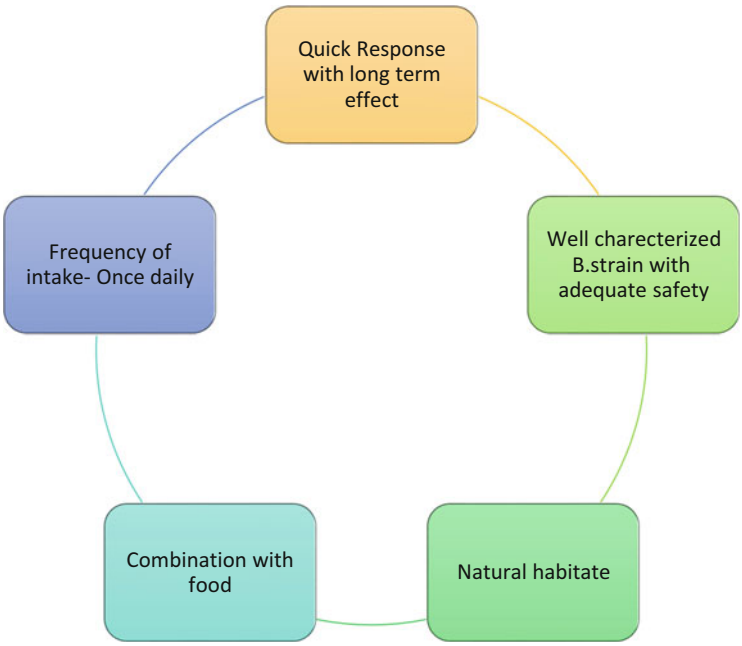

Fig. 15.1 Probiotics human application requirements (Ortwin 2005)

15.3 Market Trend

Functional foods are the food enriched with ingredients for producing health benefit that was initiated in Japan in last 1980s (Sanders 1998b). The global market of these foods grew rapidly due to the increase in consumer interest in the ability of this fortified food to help prevent many diseases. Probiotics in the functional food improved gut health by improving lactose intolerance in lactose intolerant individual, treatment of diarrhea and food allergy or for improved resistance to the pathogenic bacteria. The probiotic containing dairy products such as yoghurts have experienced rapid growth (Jon 2008). The new focus in probiotics containing food products include osteoporosis, improvement in immunity, protection against carcinogens, cholesterol lowering, heart disease, and general health (Onishi et al. 2003; Jayaram et al. 2016). The increasing worldwide awareness in the consumers regarding health concerns motivated researchers to explore probiotic applications in various other potential clinical/health avenues (Saxelin 2008). Figure 15.2 depicts the patent application trend for the probiotics globally. When we bifurcate it, it seems that more than 2500 applications have been filed at the WIPO office only (Baltatzis and Eckhouse 2019).

A report from the survey predicts the global probiotic market to reach US $ 74 to 77 billion by the end of 2025 as compared to $ 52.55 billion in 2018 (www.

Table 15.1 Probiotic strains and their commercial source (Yeung et al. 2002; Ansari 2019; Fijan 2014)

Probiotic strain	Source
Bifidobacterium breve	Yakult (Japan)
B. lactis Bb-12 Chr	Hansen, Inc. (Denmark)
B. lactis FK120	Fukuchan milk (Japan)
B. lactis HN019 (DR10)	New Zealand Dairy Board. (N.-Z.)
B. lactis LKM512	Fukuchan milk (Japan)
B. longum BB536	Morinaga Milk Industry Co., Ltd. (Japan)
B. longum SBT-2928	Snow Brand Milk Products Co., Ltd. (Japan)
B. species 420	Danlac (Canada)
Enterococcus faecium SF68	Cerbios Pharma (Switzerland)
Lactobacillus acidophilus CK120	Matsutani Chemical Product (Japan)
Lb. acidophilus NCFB 1748	Arla (Sweden)
Lb. acidophilus 145	Danlac (Canada)
Lb. acidophilus 74-2	Danlac (Canada)
Lb. acidophilus DDS-1	Nebraska Cultures, Inc. (USA)
Lb. acidophilus LA-1	Chr. Hansen, Inc. (USA)
Lb. acidophilus LB	Lacteol Laboratory (France)
Lb. acidophilus	NCFM® Rhodia, Inc. (USA)
Lb. acidophilus R0011	Institut Rosell (Montreal, Canada)
Lb. acidophilus SBT-2062	Snow Brand Milk Products Co., Ltd. (Japan)
Lb. bulgaricus 1261	Danlac (Canada)
Lb. casei 01	Chr. Hansen (Denmark)
Lb. casei 744	Nutricia (The Netherlands)
Lb. casei CRL431	Chr. Hansen (Denmark)
Lb. casei Imunitass (Defensis, DN114, DN-014001)	Danone (France)
Lb. casei Shirota (YIT 0918)	Yakult (Japan)
Lb. casei var. rhamnosus (Lactophilus)	Laboratoires Lyocentre (France)
Lb. crispatus CTV05	Gynelogix, Colorado (USA)
Lb. delbrueckii subsp. bulgaricus 2038	Meiji (Japan)
Lb. fermentum RC-14	Urex Biotech (Canada)
Lb. helveticus CK60	Matsutani Chemical Product (Japan)
Lb. johnsonii La-1 (Lj1)	Nestec Ltd. (Switzerland)
Lb. paracasei CRL 431	Chr. Hansen, Inc. (Denmark)
Lb. paracasei F19	Arla Dairy (Sweden)
Lb. plantarum 299V	Probi AB (Sweden)
Lb. plantarum ATCC 8014	MicroBioLogics (MBL), USA
Lb. plantarum L2-1	Danlac (Canada)
Lb. reuteri MM53	BioGaia (Sweden)
Lb. reuteri SD2112 (MM2)	BioGaia (USA)
Lb. rhamnosus 1091	Danlac (Canada)

(continued)

Table 15.1 (continued)

Probiotic strain	Source
Lb. rhamnosus 271	Probi AB (Sweden)
Lb. rhamnosus ATCC 7469	MicroBioLogics (MBL) (USA)
Lb. rhamnosus GG (ATCC 53103)	Valio Dairy (Finland)
Lb. rhamnosus GR-1	Urex Biotech (Canada)
Lb. rhamnosus LB21	Essum AB (Sweden)
Lb. rhamnosus LC-705	Danlac (Canada)
Lb. rhamnosus R0052	Institut Rosell (Canada)
Lb. rhamnosus VTT E-97800	Research strain VTT, Finland
Lb. salivarius UCC118	University College Cork (Ireland)
Lactococcus lactis L1A	Essum AB (Sweden)
Streptococcus thermophilus 1131	Kenko-dontokoi (Japan)
St. thermophilus F2	Danlac (Canada)

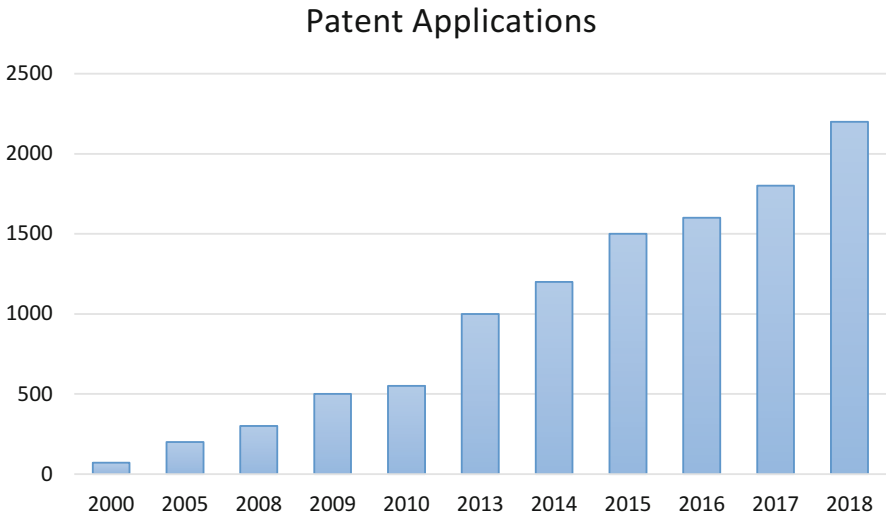

Fig. 15.2 Patent application trend for probiotics

mordorintelligence.com/industry-reports/probiotics-market). The market size of lactobacillus strains was valued at US $ 1.2 billion in 2017 while increase in 6% until 2021 is predicted for the market size of bifidobacterium (https://www.prnewswire.com/news-releases/probiotics-market-worth-us74-69-bn-at-7-3-cagr-by-2025-exclusive-report-by-fortune-business-insights-300843432.html). The bacillus strain market size may exceed US $ 180 million by 2024 (Dixit et al. 2016). Most common health disorders are often related to the change in the lifestyle. Increased access to information has instigated conscientious customers to be more knowledgeable than ever, embracing probiotic for improving their health. Microbial genus of lactobacillus, bifidobacterium, saccharomyces dominated the global market though many

other genera like streptococcus, enterococcus, propionibacterium, and bacillus are successfully scaled up commercially for wide variety of human application as depicted in the Table 15.2.

Wide varieties of probiotics are given as formulations in hard gelatin or vegetable capsules, tablets with or without enteric coating, chewable tablets, and powder sachets. Formulations may also contain other supplementary active components including vitamins and prebiotics. Probiotics are also sold in the suspensions for the ease of administration to the infants. Further, for the treatment of acute diarrhea probiotics are also combined along with the oral rehydration sachets. The consumer demands for probiotics containing nondairy products like juice, chocolate-based products, beverages, vegetables, cereals-based products, processed meat, etc. have also increased (Stanton et al. 2001). From the publication trend it appears the late 1980s and early 1990s had some activity in this field but real pursuit for building IP around probiotics happened only more recently in the last decade and has been quite consistent since (Fig. 15.2). In the year of 2010, almost 1200 patents were published in the probiotic field, which is nearly 400 in 1950 (Technology Insight Report 2011).

15.4 Patent Scenario

Every day seems to bring exciting research about the important role of probiotics in human health. From Consumer Point of view, probiotic usage is not only limited for digestive health, but expanded as immunity booster, for inflammatory conditions, women health improvement, CNS and Brain related ailment, oral health, and cosmetic and personal care. Consumers are now ready to loosen their pocket for premium branded ingredients, if they have gained trust on it. A number of natural product companies have capitalized on the need for innovative products that consumers can trust by protecting these products with intellectual property (Baltatzis and Eckhouse 2019). As probiotics paved their importance in health and well-being, the patent family for the same has now reached over 10,000 throughout the world. The increasing rate of filing shows that there are many inventions that continue to be discovered (Figs. 15.3 and 15.4) (Boschloo 2011). We have conducted patent search using Google Patent, WIPO, patent storm, etc. and summarized our findings for the probiotics patent in Table 15.3. Most of the patent applications (Patent Cooperation Treaty, PCT) have been published by the World Intellectual Property Organization (WIPO) (Baltatzis and Eckhouse 2019).

15.5 Probiotics as Pharmaceuticals

Hippocrates, (400 BC) defined in his prophecy that a bad digestive system is the culprit for the diseases in human. Antibiotic-resistant bacterial strains were developed due to the irrational use of the antibiotics, which is the point of trigger. The mechanism of action of the probiotics is depicted in Fig. 15.5.

Table 15.2 Marketed probiotics for veterinary use (Protexin 2019; Tomlyn 2019; Vetriscience 2019; Biomen 2019; Refit 2019)

Sr. no.	Target animal	Brand name	Company	Probiotics/prebiotics	Applications
1	Horse	Gut Balancer® Recover Aid®	Protexin® Equine premium	*Saccharomyces cerevisiae* Fructo oligosaccharide	Gut Health, Encourage appetite and aid recovery
2	Horse	Acid Ease®	Protexin® Equine premium	*S. cerevisae* Fructo oligosaccharide Lignocelluloses	Calm Acidity and Digestibility enhancers
3	Canine	Tomlyn pre and probiotic dog supplement	Tomlyn®	*Enterococcus faecium Lactobacillus casei Lactobacillus acidophilus Lactobacillus plantarum Bifidobacterium breve* Fructo oligosaccharide	Digestive and bowel Health
4	Feline	Perio support®	Verti Science labs	*Enterococcus faecium Lactobacillus acidophilus* Natural zeolites, cranberry extracts, Yucca schidigera extracts	Clean teeth and fresh breath
5	Goat	Probiotic powder	Goats prefer	*Lactobacillus acidophilus*	Maintain normal digestion and appetite
6	Poultry Povine, Fish, Shrimps	Mycofix®	Biomin®	T. mycotoxinivorans Biomin®	Protects animal health by deactivating mycotoxins found in contaminated feed
7	Bovine, Aquaculture animals	Levabon® Rumen E Levabon® Aquagrow E	Biomin®	*Saccharomyces cerevisiae,* culture polysaccharides like glucan, mannan	Growth of fiber digesting bacteria in bovine, promote digestion, immune modulation
8	Poultry Birds	FloraZone	Refit Animal Care	*Lactobacillus acidophilus* Bifidobacterium *Lactobacillus rhamnosus Saccharomyces boulardii*	Exclusion of Pathogenic bacteria, favorable pH in small intestine, prevents bacterial infections,

(continued)

Table 15.2 (continued)

Sr. no.	Target animal	Brand name	Company	Probiotics/prebiotics	Applications
				Bacillus subtilis *Lactobacillus plantarum*	improves growth and performance

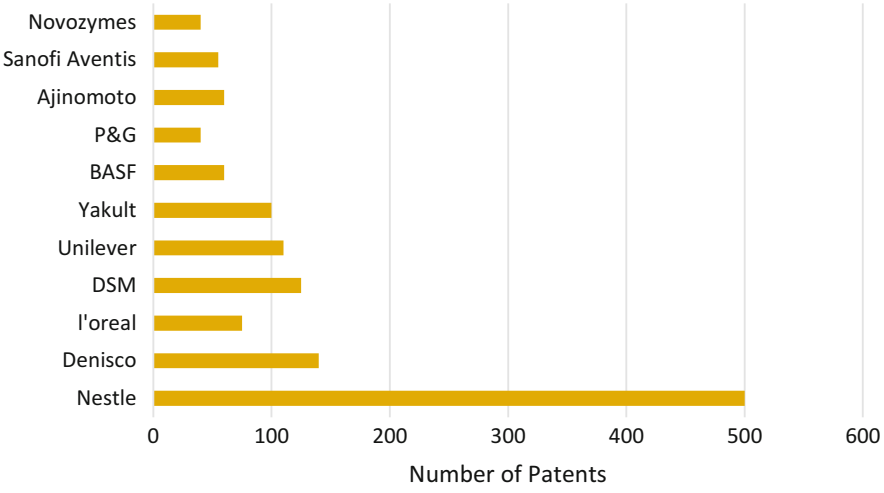

Fig. 15.3 Top assignees of patent in probiotic (modified from the data of Baltatzis and Eckhouse 2019; Technology Insight Report 2011)

Application of probiotics and their antimicrobial metabolites such as bacteriocins is a novel strategy in the treatment and prevention of gastrointestinal infections. Certainly, the medical use of probiotics will continue to increase, and more probiotic products will be registered as drugs. In particular, genetically modified bacteria will find their way into drugs, and manufacturing plants that specialize in production of both drugs and genetically modified organisms will need to be established. Some of such applications have been depicted in the Fig. 15.6. Beneficial health effects of probiotics and their mechanism of actions to achieve the same is summarized in the Table 15.4.

15.6 Cosmetic and Personal Care

The probiotic food supplement are the functional food intended to alter, modify and restore the preexisting intestinal flora. Much of the studies in the research and commercial products have focused on the human gastrointestinal tract however in the last few years, symbiotics probiotics research and application have been

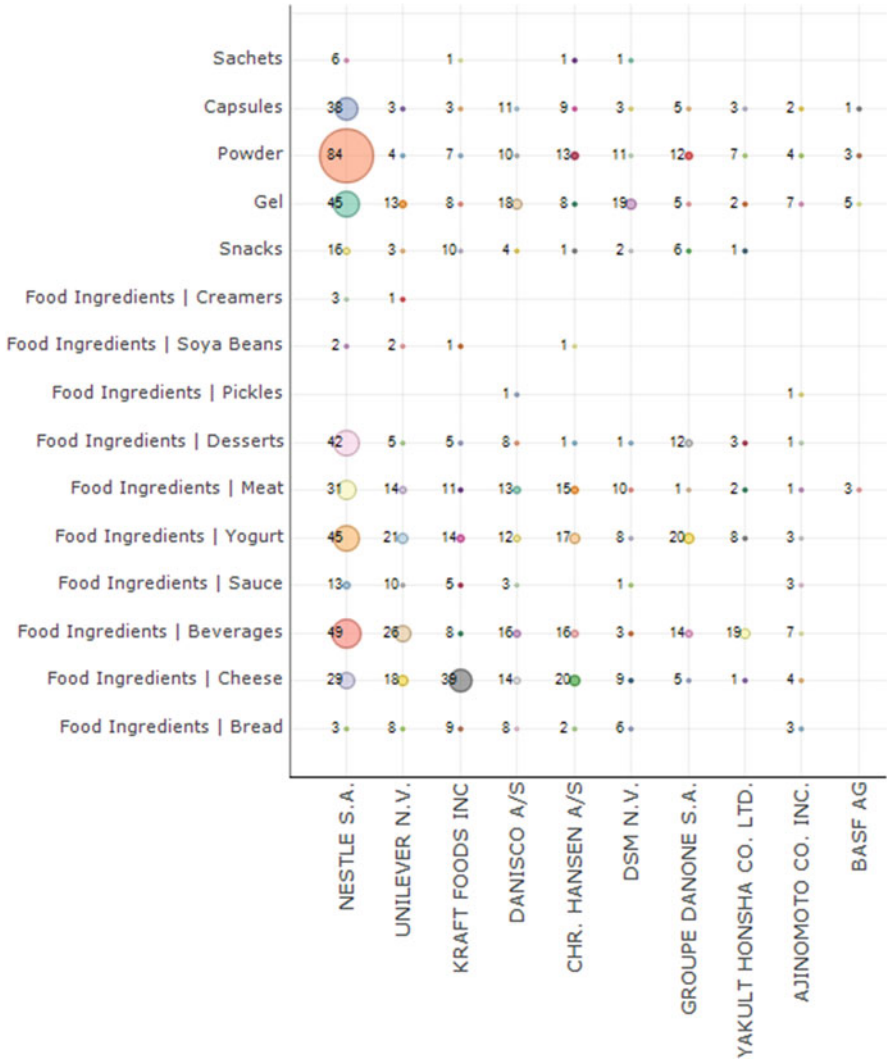

Fig. 15.4 Inventions across different formulations for probiotics with different companies (adopted from Technology Insight Report 2011)

expanded to other physiological systems like cosmetic and personal care products (Farmer and Mikhail 1998). As the horizon of the studies on prebiotic continues to grow the potential applications are developed in the personal care products like lotions creams and oral care products. For years, the skin care industry has been investigating probiotics to enhance the functionality and beauty of the skin and researchers have been assessing whether probiotic could be used to treat certain skin conditions (Al-Ghazzewi and Tester 2014). There are also reports indicating the

Table 15.3 Patent scenario for probiotics at a glance

Sr. No.	Patent No.	Year	Details of patent	*Microorganism*	Assignee
1	WO2019060661A1	2019	Probiotic compositions for production of dopamine	*E. faecium*	Mark Lyte, Daniel Nicholas VILLAGELIU
2	TWI659745B	2019	Probiotics oral health care method using sugar	*Streptococcus mitis*, *Streptococcus sanguinis*, *Veillonella parvula*, A group of *Streptococcus gordonii*, and *Actinomyces naeslundii*	Taiwan
3	KR20190067527A	2019	A Bacillus probiotics mixture for preventing or treating obesity, diabetes and fatty liver and uses thereof	*Bacillus sonorensis* (*Bacillus sonorensis*) JJY12-3 strain (Accession No: KCTC13405BP), *Bacillus paralicheniformis* (*Bacillus paralicheniformis*) JJY12-8 strain (Accession Number: KCTC13406BP), *Bacillus sonorensis* JJY13-1 (Accession No.: KCTC13407BP), *Bacillus sonorensis* JJY13-3 (Accession No.: KCTC13408BP), *Bacillus sonorensis* JJY13-8 strain (Accession No.: KCTC13409BP)	Kim Min-seok, Park Ha-ryeong, Kim Bo-bae, Joseph, Holzafel Wilhelm, Kim Do-young, Lee Eun-ju, Kim Na-ri, Park Seul-ki, Park Ho, Choi Bo-hwa
4	ES2567404T3	2019	Probiotics to influence the metabolism of fats and obesity	*Lactobacillus casei* F19 (LMG P-17806), *Lactobacillus acidophilus* (NCFB 1748), and *Bifidobacterium lactis* Bb12 (DSM 15954)	Kajsa Ohlson, Margit Mahlapuu, Ulla Svensson
5	JP6033998B2	2019	Storage and delivery of probiotics	Lactic acid bacteria, Bifidobacterium, etc.	Sanguansri, Razz, Augustin, Mary Ann, Crittenden, Ross
6	DE60133581T2	2019	*Lactobacillus casei* KE01 stem derived probiotic compounds	*Lactobacillus casei* KE01	A. Satyanarayan Diamond Bar NAIDU

(continued)

Table 15.3 (continued)

Sr. No.	Patent No.	Year	Details of patent	*Microorganism*	Assignee
7	JP2019510036A	2019	A detergent composition comprising probiotics/prebiotics active ingredient	Spore-forming bacteria, e.g., *Bacillus coagulans*, *Bacillus subtilis*, *Clostridium* or *Lactobacillus sporogenes*	Sarah Gantz, Sarah Gantz, Amanda Copland, Amanda Copland, Carrie Ann Zapka, Carry Ann Zapka
8	JP2019502737A	2019	Probiotics for use as anti-inflammatory agents in the oral cavity. It can be used for the gingivitis and/or periodontitis	*Lactobacilli*, e.g., *L. acidophilus* or *L. brevis*	Marx Rudolph Getz, Marx Rudolf Getz, Kelstein Holmglen, Kelstein Holmglen, Niklas Larson, Niklas Larson, Bernd Fiebich, Bernd Fiebich, William Wade, William Wade
9	CN108753677B	2019	A kind of multifarious high-activity probiotics composition for improvement of infant's intestinal flora. It has good heat resistance, acid resistance, and drug resistance, has broad application prospects in fields such as food, medicine, feed, environmental protection	*Lactobacillus rhamnosus* 2–8 parts and 2–8 parts of *Lactobacillus fermenti* CECT5716	Zhang Wei, Liu Haixia, Hao Ran (Zhongke Yikang (Beijing) Biotechnology Co., Ltd.)
10	CN208542126U	2019	A kind of probiotics solid beverage mixing arrangement	Lactic acid bacteria, Bifidobacterium, etc.	Yu Xueping (Shan Enkang Biotechnology (Suzhou) Co., Ltd.)
11	CN208581827U	2019	A kind of raw material granulation equipment of probiotics chewable tablets	Lactic acid bacteria	Zhang Jianming, Xiao Dexun, Xie Yan, Li Yongkai, He Dapeng, Zhao Mingzhe (Jiangxi Shumet Pharmaceutical Co., Ltd.)
12	CN104957255B	2019	Brown probiotic yogurt and preparation method thereof. The prebiotic bacterial content of the Yoghurt reaches 10^{10} Cfu/100 g or	*Bifidobacterium, thermophilic, Lactobacillus lactis, Lactobacillus acidophilus, Lactobacillus casei,*	Miao Junli, Guo Benheng, Liu Zhenmin, Xu Zhiyuan, Ying Jie, Shen Ling, Wang Hao, Liao Wenyan, Han Mei, Lü Changyong

			more, has both the advantage of brown fermented milk and probiotics fermentation cream	*Lactobacillus rhamnosus* and *leuconoid (Leuconostoc)*	
13	EP2837292 A1	2019	Synbiotic food composition containing tagatose and probiotic lactic acid bacteria which promotes active intestinal growth of lactobacillus species	*Lactobacillus casei* strain or *Lactobacillus rhamnosus* strain as a probiotic, and tagatose as a prebiotic	Ji Hoon KohYoung Jae KimSeung Won Park
14	WO1998047374A1	2019	Topical use of probiotic bacillus spores to prevent or control microbial infections	*Bacillus coagulans, Bacillus subtilis, Bacillus laterosporus*, and *Bacillus laevolacticus*	Sean Farmer Robert J. Mikhail
15	KR20180135504A	2018	Method of fermented white ginseng composite using probiotic strain	*Lactobacillus* sp.	Seon-gyun Ha, Yu-jin Ha, Seul-ki Kim, Jung-gu, Tae-hoon Ko, Kyu-tae Cho
16	WO2017207371A9	2018	Bacillus licheniform is strain with probiotic activity	*Bacillus licheniformis*	Daniel Petri, Stefan Pelzer, Jessica kleinböltiNG, Stella Molck, Maike kipker, Claudia borgmeier, Sandra HERBOLD, Guido Meurer, Rose Whelan, Kiran doranalli
17	WO2018174938A1	2018	Stable dry powders and emulsions containing probiotics and mucoadhesive protein	*Lactobacilli* and *bifidobacteria*, and other beneficial bacterial species, such as *Streptococcus thermophilus*	Philip J. Bromley
18	KR101853603B1	2018	Composition containing of probiotics for using alcohol or acetaldehyde dehydrogenase activity	*Lactobacilli*, e.g., *L. acidophilus* or *L. brevis*	Myung Joon Jung

(continued)

Table 15.3 (continued)

Sr. No.	Patent No.	Year	Details of patent	*Microorganism*	Assignee
19	KR101839375B1	2018	Bacillus amyloliquefaciens SRCM 100730 strain having antimicrobial activity and probiotics properties and uses thereof	*Bacillus amyloliquefaciens* SRCM 100730	Uhm Jung-sun, Ryu Myung-sun, Cho Seung-hwa, Yang Hee-jong, Jeongsooji, Jung Sung-yeop, Jeong Do Yeon (Fermentation Microbial Industry Promotion Agency)
20	KR101927859B1	2018	Method for improving the stability and coating efficiency of probiotics using ultrasonic wave after freeze-drying and food composition containing freeze-dried powder of probiotics prepared thereby as effective component	The probiotics (Probiotics) is *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus gasseri*, *Lactobacillus delbrueckii*, *eseueseu blood Bulgaria kusu* (*Lactobacillus delbrueckii* ssp. *bulgaricus*), *Lactobacillus helveticus* (*Lactobacillus helveticus*), Lactobacillus buffer momentum (*Lactobacillus fermentum*), Lactobacillus para casei (*Lactobacillus paracasei*), Lactobacillus ramno suspension (*Lactobacillus rhamnosus*), *Lactobacillus reuteri, Lactococcus lactis*, Streptococcus thermo-pillar's (*Streptococcus thermophilus*), *Bifidobacterium ronggeom* (*Bifidobacterium longum*), *Bifidobacterium bonus* (*Bifidobacterium bifidum*), *Bifidobacterium breve, Bifidobacterium animalis* ssp. *Lactis*	Lee Myung-hee, Jeong-sung, Lee Ho-jin, Hong Dong-ki, Na Kuk-nam, Lee Jae-ho, Choi Il-dong, Lee Jung-yeol, Shim Jae-heon

21	KR101932955B1	2018	Probiotics Composition for Improving Intestinal Microbial Flora. It can be used for the colon cancer patient who underwent the anterior resection surgery	*Bifidobacterium* (*Bifidobacterium* spp.)	Joo Yeon Kim, Soo Dong Park, Jung Woong Jung, Jae Joong Shim, Sung Sik Jang, Jung Yeol Lee, Jae Heon Shim
22	CN106890197B	2018	Probiotic composition for rhinitis, Asthma, allergic dermatitis or for treating cranial nerve diseases or as an anti-cancer or in nasopharyngeal carcinoma or anti-cold prevention or in the treatment of respiratory or and other infectious diseases	Lactic acid bacteria, e.g., enterococci, pediococci, lactococci, streptococci or leuconostocs	Du Bin (Chengdu Yizhi Biological Technology Co., Ltd.)
23	EP1607096B1	2018	A nutraceutical composition containing a large number of live milk enzymes (human and coated) with aloe and carrageenans, in combination with suitable prebiotic substances (arabinogalactan), which consequently possesses synbiotic activity	*Lactobacillus sporogenes*	Carlo Angelloti
24	US20080305089	2018	A composition which includes naturally occurring, non-transgenic isolated bacteria from the group of Lactococcus and Acinetobacter for the prevention and/or treatment of allergic or chronic inflammatory disorders	Lactococcus and Acinetobacter	Ruhr-Universitat Bochum Forschungszentrum Borstel
25	CN106857856A	2017	Complex probiotics product beneficial for respiratory system	*Streptococcus thermophiles* and *Lactobacillus rhamnosus*	Wang Yangguang

(continued)

Table 15.3 (continued)

Sr. No.	Patent No.	Year	Details of patent	*Microorganism*	Assignee
26	WO2017207372A1	2017	Bacillus subtilis strain with probiotic activity	*Bacillus subtilis* DSM 32315	Daniel Petri, Stefan Pelzer, Jessica kleinböltiNG, Stella Molck, Maike kipker, Claudia borgmeier, Sandra herbold, Guido Meurer, Rose Whelan, Kiran doranalli
27	US20140205581A1	2017	Formulations of Saccharomyces cerevisiae var. boulardii and the enzyme superoxide dismutase to control obesity	Ignazio CastagliuoloPaola BrunImmacolata BusielloNiccolo Miraglia	Saccharomyces cerevisiae var. boulardii
28	US20100183559	2017	Genome of a probiotic Bifidobacterium longum and genes encoded by the genome	*Bifidobacterium longum*	Douwe Van SinderenJun XuWenzhu (Steven) ZhaoRaymond A. GrantSong YuliCharles BascomDuane Larry CharbonneauLiam O'Mahony
29	CN105559064A	2016	The five-alga probiotic enzyme tablets are prepared from the following raw materials in parts by mass: 10–20 parts of chlorella vulgaris, 1–10 parts of Dunaliella salina, 1–10 parts of euglena, 1–10 parts of red algae, 10–20 parts of spirulina and 10–20 parts of probiotic powder. The five-alga probiotic enzyme tablets provided by the invention adopt the chlorella, Dunaliella salina, red algae, spirulina and euglena for complementation in nutrition, and obtain trace elements	*Bifidobacterium adolescentis, Bifidobacterium longum, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus, Saccharomyces cerevisiae, Lactobacillus subtilis* any one or any two or more thereof	Yu Haohao, Li Chunling

			easier to absorb by a human body through probiotic fermentation, thereby helping to reinforce the immunologic function of the human body, resist virus infection and proliferation, inhibit cancer cell proliferation, repair the damage of an organism quickly, remove vivotoxin, suppress blood pressure and blood sugar rise, reduce the serum cholesterol content, and thus improve the comprehensive conditioning of the human body		
30	CN105647721A	2016	Probiotic dry red wine and its preparation method	*Lactobacillus plantarum, Bifidobacterium bifidum, Bifidobacterium infantis, Bifidobacterium longum, B. breve, B. adolescentis, Bifidobacterium lactis, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus rhamnosus, Streptococcus thermophilus*	Li Jianshu, Li Zheng
31	US20130302299A1	2016	A novel Bacillus sp. 2-4 (KCCM11107P) strain having probiotic activity. This strain has an antibacterial activity against various fish pathogenic bacteria and they also secrete enzymes which helps in digesting fish food	*Bacillus* sp. 2-4 (KCCM11107P) strain	Young Ok KimKyung Kil KimBo Hye NamSang Jun LeeDong Gyun KimHyon Sob HanSi Yong YANGHee Jeong KongWoo Jin KimBong Seok Kim

(continued)

Table 15.3 (continued)

Sr. No.	Patent No.	Year	Details of patent	*Microorganism*	Assignee
32	EP2990045A1	2016	Invention relates to a method for reducing abdominal pain in a postmenopausal woman, the method comprising administering a probiotic product comprising Bifidobacterium animalis subsp. lactis to the postmenopausal woman	*Bifidobacterium animalis* subsp. lactis	Dorte EskesenLillian JespersenCathrine Melsaether MorbergBirgit Michelsen
33	EP2477637 A1	2016	This patent relates to a selected probiotic strain of Lactobacillus rhamnosus and its uses in the nutritional, cosmetic, and/or medical fields and the patent is about its anti-inflammatory activity	*Lactobacillus rhamnosus*	Giammaria GiulianiAnna BenedusiAntonio Mascolo
34	US20160151434A1	2016	A method of treating intestinal disorder by administering an effective dose of Lactobacillus plantarum	*Lactobacillus plantarum*	Il Young KwackSe Jin YouTae-Hun ParkBum Jin LeeKye Ho ShinJin Oh ChungJun Cheol Cho
35	CN105123931A	2015	Probiotic foodstuff and its preparation method. The prepared probiotic foodstuff has fine mouth feel, uniform texture, stable state, natural quality, a great number of live bacteria, strong functionality and long shelf-life, wherein the content of the live bacteria is 1.75×10^{11} to 2.55×10^{11} CFU/g, and the shelf-life is 30–36 months at normal temperature	*Lactobacillus plantarum*	Shao Suying

36	CN104586737A	2015	Probiotic whitening recovery mask essence and its preparation method	*Lactobacillus*	Xie Shuchun, Yang Kai
37	CN105192832A	2015	Composition of probiotic fermented horseradish tree leaves as well as preparation method and application of composition. It can be used for reducing the contents of total cholesterol (TC) and triglyceride (TG) in blood serum, and improving the content of high density lipoprotein cholesterol (HDL-C) in the blood serum; the composition of the probiotic fermented horseradish tree leaves, disclosed by the invention, has a function of lowering a blood fat level	*Lactobacillus* and *Bifidobacterium*	Wu Bingxin, Sun Yulin, Yan Xinhong, Zhai Zongwu, Lu Jichen, Lu Mei
38	CN104770816A	2015	Watermelon juice probiotic fermented beverage and its preparation method	Probiotic Bifidobacterium strains used (*Bifidobacteria*), a number of lactobacilli and streptococci, which are commonly *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus bulgaricus*	Ningxia Hengrui Biological Food Technology Co., Ltd.
39	CN104543679	2015	Preparation method for probiotics. It will inhibit the growth of harmful fungi and gram negative bacteria of the intestine	*Lactobacillus kefir* MA2	Shaoxing Shangyu Hongwei Technology Transfer Service Co., Ltd.
40	US 20140234260 A1	2015	Composition useful for the treatment and/or prophylaxis of chronic disorders associated with the presence in the gastrointestinal tract	*Clostridium* and *Bacteroides*	Thomas Julius Borody

(continued)

Table 15.3 (continued)

Sr. No.	Patent No.	Year	Details of patent	*Microorganism*	Assignee
			of a mammalian host of abnormal or an abnormal distribution of microflora, which comprises a viable non-pathogenic or an attenuated pathogenic Clostridia		
41	US9011838B2	2015	Administering a lactic acid-producing bacterium at a relatively low dosage in combination with a lactate utilizing bacterium, followed by administration of the lactic acid-producing bacterium at a relatively high dosage to the animal. These help to achieve pre-harvest food safety and enhance feed performance while keeping the total cost relatively low	*Lactobacillus acidophilus*/animalis *Propionibacterium freudenreichii*	Douglas Ware Peter Anderson
42	US9011877	2015	Methods of isolating strains of Lactic acid bacteria for giving as a direct fed to animals are provided and the method of preparing this direct fed microbial is also provided	Lactic acid bacteria (*Lactobacillus acidophilus* strain P1B c6 (NRRL B-50103), *Lactobacillus salivarius* strain o246e 33w (NRRL B-50102), *Pediococcus acidilactici* strain o246e 42 (NRRL B-50171), and *Pediococcus acidilactici* strain P1J e3 (NRRL B-50101))	Mari Ellen Davis, Waukesha, WI (US); Joshua M. Rehberger, Milwaukee, WI (US); Charles Maxwell, Springdale, AR (US); Thomas G. Rehberger, Wauwatosa, WI (US); Mike King, Oak Creek, WI (US)
43	US9144588	2015	Bacillus subtilis isolated from corn is said to be having antimicrobial activity and so these could be used to maintain a healthy gastrointestinal flora	*Bacillus subtilis*	Sean FarmerRobert J. Mikhail

44	US20140023620	2015	Probiotic compositions of two probiotic bacteria, which includes Streptococcus salivarius and Lactobacillus bacteria for improving oral health	*Streptococcus salivarius* K12® (BLIS K12®) and at least a *Lactobacillus bacteria*	Natalya Ioudina
45	WO/2015/090349	2015	The present invention provides combinations of probiotics, Longum BB536/BL-999 with L. Rhamnosus LPR/LGG or B. Longum BB536/ BL-999 with L. Paracasei ST-11, for administration to expectant females and/or lactating mothers (two weeks before delivery), and optionally to their progeny for the reduction or prevention of the development of allergy in progeny	*Lactobacillus Rhamnosus* LPR (LGG) and *Bifidobacterium Longum* BL999 (BB536)	Florence Rochat
46	EP 2467031 A1	2014	Nutritional composition comprising Lactococcus strains or probiotic is provided for reducing the symptoms of allergies in different groups of patients	*Lactococcus lactis*	Nestec SA
47	US8697055B2	2014	Use of one or more species or strains of lactic acid-producing bacteria, preferably strains of Bacillus coagulans, for the control of gastrointestinal tract pathogens, including antibiotic-resistant gastrointestinal tract pathogens, and their associated diseases	*Bacillus coagulans*	Sean Farmer

(continued)

Table 15.3 (continued)

Sr. No.	Patent No.	Year	Details of patent	*Microorganism*	Assignee
48	US20040202749	2014	Mycological properties of Bifidobacterium longum	*Bifidobacterium longum*	Kazuyoshi SotoyamaAkinori HiramatsuJin-zhong XiaoNoritoshi TakahashiShizuki KondoTomoko YaeshimaSachiko Takahashi
49	EP1824500 A1	2013	This patent is about the probiotic bacterial strain Lactobacillus plantarum, Lactobacillus crispatus, and Lactobacillus gasseri which is having the ability to colonize the human vagina. It is used as a medicament, in food or in a pharmaceutical product	*Lactobacillus gasseri*	GÖran MOLINSiv AHRNéBengt JeppssonAlejandra VasquezAnna Berggren
50	US8460917B2	2013	Novel Lactobacillus strains like Lactobacillus rhamnosus strain Lbp PB01 which alone or in combination with Lactobacillus gasseri strain Lba EB01 (DSM 14869) can be used as probiotics	*Lactobacillus rhamnosus* strain Lbp PB01	Gunnar BrøndstadErik Brandsborg
51	US8557320 B2	2012	Nutritional composition having a prebiotic which increases the population and species of beneficial bacteria and also slows the fermentation of prebiotics within the gut	*Bifidobacteria* spp., *Lactobacillus* spp	Bryon W., PetschowRobert J., McMahonGlenn R., GibsonRobert A., RastallRenia, GemmellMaria, SaarelaAnna-Marja Aura
52	EP2519108 A1	2012	The use of targeted antibiotics with probiotic formulations for treatment and prophylaxis of inflammatory bowel diseases	*Bifidobacterium bifidum*; *Bifidobacterium breve*; *Bifidobacterium infantis*; *Bifidobacterium longum*;	Ira Milton Trachtman

				Lactobacillus acidophilus; *Lactobacillus bulgaricus*; *Lactobacillus paracasei*; *Saccharomyces boulardii*	
53	EP2318513A1	2012	The invention is about novel, probiotic, anti-inflammatory strains of Bifidobacterium longum, their use for treatment of diseases, and for preparation of human or pet food or pharmaceutical compositions	*Bifidobacterium longum*	Jens KildsgaardThomas Dyrmann LeserThomas GunnarssonMette WeiseDitte Marie FolkenbergThomas JanzenBenedicte Flambard
54	EP 2519108A1	2012	Methods for treating inflammatory bowel disease involve the use of targeted antibiotics in combination with probiotic formulations. which reduces use of immunosuppressants in the treatment	*Bifidobacterium bifidum*; *Bifidobacterium breve*; *Bifidobacterium infantis*; *Bifidobacterium longum*; *Lactobacillus acidophilus*; *Lactobacillus bulgaricus*; *Lactobacillus paracasein*; *Saccharomyces boulardii*	Ira Milton Trachtman
55	US 2011/0052538 A1	2011	Composition of Lactobacillus strain and a non-digestible oligosaccharide which provides gastrointestinal health	*Bifidobacterium*, *Streptococcus*, *terococcus*, *Lactococcus*, *taphylococcus*, *Peptostreptococcus*, *Lactobacillus*, *Saccharomyces*, *Propionibacterium*	Ian Lewis BrownAnne M. BirkettRichard Le LeuGraeme P. Young
56	WO2011092261 A1	2011	Probiotic composition with Lactobacillus plantarum CECT 7484, Lactobacillus plantarum CECT 7485, and Pediococcus acidilactici CECT 7483 for treatment of gastrointestinal diseases	*Lactobacillus plantarum* CECT 7484, *Lactobacillus plantarum* CECT 7485, and *Pediococcus acidilactici* CECT 7483	Jordi Espadaler MazoJordi CUÑÉ CASTELLANA

(continued)

Table 15.3 (continued)

Sr. No.	Patent No.	Year	Details of patent	*Microorganism*	Assignee
57	US8058051	2011	Bifidobacterium bifidum has an effect of killing Helicobacter pylori and it also shows high survivability even in the case of being stored in a fermented milk food or drink under aerobic condition	*Bifidobacterium bifidum*	John MacsharryLiam O'mahonyDavid O'sullivanBarry Kiely
58	US 7361497 B2	2008	Isolation characterization of probiotic bacteria and it also describes treating salmonellosis in food production animals	*Bacillus circulans* ATCC PTA-5614,5615,5616	Michael P. DoyleMichelle D. Danyluk
59	US 7427397 B2	2008	Probiotic Propionibacterium strains and their use in preparation of probiotic supplements and foods are described. They have also said about preparation of vaccines for use in protecting patients from infectious diseases, in particular tuberculosis	*Propionibacterium jensenii* 702	Michelle Catherine Adams, Yang Huang
60	WO/2008/ 117267A2	2008	Immunomodulatory functions of probiotic Bifidobacterium strain	*Bifidobacterium strain*	John MacsharryLiam O'mahonyDavid O'sullivanBarry Kiely
61	US20070098744 A1	2007	Probiotic enterococci for improved immunity	*Enterococcus species*	Ruth Knorr, Christoph Cavadini, Jalil Benyacoub, Ebenezer Satyaraj
62	CA 2689862 A1	2007	Probiotic composition of propionibacterium and its use as a food supplement for normalizing the gastrointestinal flora	*Propionibacterium, Lactobacterium, Bifidobacterium, and Streptococcus*	Fabiola Masri
63	US 20060165670 A1	2006	Synbiotic combination of composition comprising a Lactobacillus strain and a non-digestible oligosaccharide	*Lactobacillus* strain NCIMB 41114	Michael BeerGlenn GibsonChristopher Smejkal

64	EP1656150	2006	Use of one strain of bacteria from Bacilli for the production of a composition intended to prevent vertebral compression syndrome in fish (Salmonid family)	*Lactobacillaceae*, *Carnobacteriaceae*, *Enterococcaceae*, *Leuconostocaceae*, and *Streptococcaceae*	Joël AUBINLaurent LabbeFrançois-Joël GATESOUPELuc Lebrun
65	EP1510135 A1	2005	To reduce the number of pathogenic Escherichia coli O157:H7 cells in feed farm animals such as cattle, Enterococcus is added to farm animal feed product	*Enterococcus*	Steven C. JohnsonWilliam P. KautzKelly F. LechtenbergJane A. Z. Leedle
66	EP0975227A1	2005	Topical use of probiotic bacillus spores to prevent or control microbial infections	*Bacillus coagulans*	Sean FarmerRobert J. Mikhail
67	US6797266 B2	2004	A new Strain of Lactobacillus casei designated KE01 possesses probiotic activity and has a in vivo anti-enteric pathogen activity	*Lactobacillus casei* strain KE01	A. Satyanarayan Naidu
68	W02005034970 A1	2004	Use of Food-grade or non-pathogenic probiotic culture to treat localized infections	*Lactococcus lactis* DPC3147, *Lactococcus lactis* 5399, *L. plantarum* DPC4922	Paul RossStephen Hallahan
69	US20030017192 A1	2003	Process for producing extended shelf-life ready-to-use milk compositions which contains probiotics by ultrapasteurizing a milk composition, cooling the composition to 20–30 °C, and then inoculating the composition with aseptically prepared probiotic cultures, which helps to improve consumers intestinal health	*Bifidobacterium genera, Lactobacillus genera*	Hanny KanafaniLorna Mize

(continued)

Table 15.3 (continued)

Sr. No.	Patent No.	Year	Details of patent	*Microorganism*	Assignee
70	WO 2002065840 A2	2002	Consumable products enriched with probiotic and a method to obtain them	*Bifidobacterium lactis*, *Lactobacillus johnsonii*, *Lactobacillus paracasei*, *Streptococcus thermophilus*	Annmarie Bengtsson-RiverosJohannes De ReuRobert Dustan WoodJohn DarbyshireHermann KnaufChristoph Cavadini
71	US 6468525 B1	2002	A probiotic formulation used as a food supplement for re-establishing beneficial bacteria to body's intestinal treat	*Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Lactobacillus salivarius*, *Bifidobacterium infantis*, and *Bifidobacterium longum*	Tommy Stanley Watson, Brenda F. Watson
72	EP1243181 A1	2002	The probiotic is a Lactococcus lactis strain (CBS108918, CBS109208) or a mutant or derivative probiotic capable of exerting a protective effect in the gastrointestinal tract	*Lactococcus lactis*	Ingeborg Marie Jacqueline, Bovee-Ouden, hoven Roelof, Van Der Meer
73	WO/1999/053932A1	2002	Streptococcus preparation for prophylaxis and/or treatment of acute otitis media (inflammation of the ear) and middle otitis (inflammation of the middle ear)	*Streptococcus sanguis*, *Streptococcus oralis*	Eva GRAHN HÅKANSSONKristian RoosStig Holm
74	EP1224867A1	2002	Characteristics of food containing Bifidobacterium	*Bifidobacterium catenulatum* or *Bifidobacterium pseudocatenulatum*	Tohru IinoFumiyasu IshikawaTakahiro MatsukiKoichiro SonoikeKaoru TochiyaEmi Yasuda
75	US 6010695 A	2000	Saccharomyces boulardii treatment to diminish enteropathogenic bacteria (campylobacter and salmonella) in poultry	*Saccharomyces boulardii* ATCC 74352	J. Eric LineNorman J. SternJ. Stan BaileyNelson A. Cox

76	WO1998000035A1	1998	Enteral compositions of Streptococcus thermophilus and Bifidobacterium longum, each at a concentration equal to or greater than 1×10^{11} CFU per gram, are useful as adjuncts for enteral formulations and also as oral nutritional supplements	*Streptococcus thermophilus* and *Bifidobacterium longum*	Renata Maria Anna Cavaliere Ved. VeselyClaudio De Simone
77	EP0573768A2	1998	Multiple bacteriocin producing lactococcus, used in foods to inhibit Lactobacillus casei and other food contaminants	*Lactococcus lactis*	Ebenezer R. VedamuthuJames T. HendersonPeter A. Vandenbergh
78	WO 1996008261 A1	1996	Composition comprising one or more probiotic microorganisms such as Bifidobacterium and a carrier (which acts as a growth medium) to transport the microorganisms to the large bowel or other regions of the gastrointestinal tract	*Saccharomyces*, *Bifidobacterium*, *Eubacterium*, *Clostridium*, *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Lactococcus*, and *Staphylococcus*, *Peptostreptococcus*	Ian L. BrownKenneth J. McnaughtRobert N. GanlyPatricia Lynne ConwayAnthony John EvansDavid Lloyd ToppingXin Wang
79	EP0508701A2	1996	A novel strain of Enterococcus faecium, deposited as NCIMB 40371, has probiotic properties and is particularly effective in treating symptoms of irritable bowel syndrome	*Enterococcus faecium*, NCIMB 40371	William Dennis AllenMargaret Anneli LinggodPhilip Porter
80	US5455028A	1995	A method of treating fungal disease in an animal, by administering an effective amount of Bacillus laterosporus strain	*Bacillus laterosporus*	Boyd J. O'Donnell
81	WO/1995/ 006736A1	1995	Two new bacteriocins from Streptococcus thermophilus and use of these in food products as an active ingredient against pathogen	*Streptococcus thermophilus*	Jacques-Edouard GermondOlivier MarcisetBeat Mollet

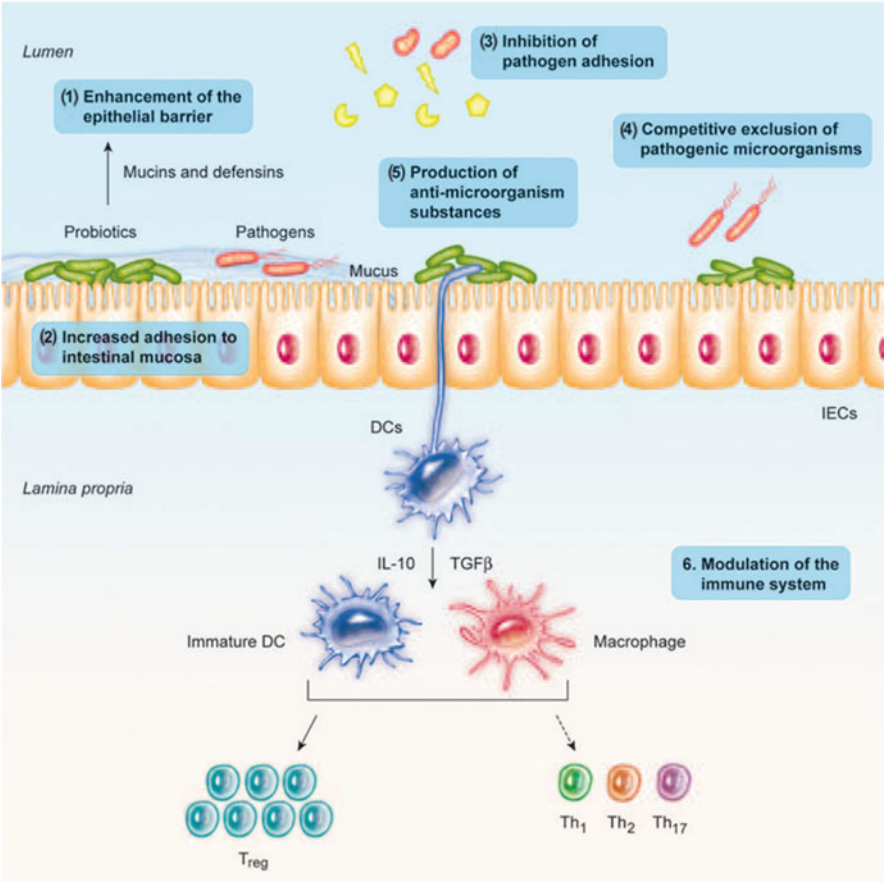

Fig. 15.5 Mechanism of action of probiotics

potential of prebiotics to compact tooth caries; it is anticipated that the trend of including probiotics in the personal care products will continue to grow (Probiotics 2019).

Altered lipid composition and organization can cause skin disease when commensal bacteria become infectious agent. The liquid composition changes during puberty stimulating lipophilic microbes such as Propionibacterium acnes to proliferate resulting in acne that affects 80% of adolescence in the USA (Avena Woods 2017). The most common form of eczema is atopic dermatitis which can affect 15–20% of the children and 1–3% of adults worldwide. Administration of probiotics revealed that in utero during pregnancy and orally in early infancy reduced the risk of developing atopic dermatitis in children from 34.7% to 28.5%. Lactobacillus and Bifidobacterium can be used as active agent for oral or tropical use in prevention and treatment of oily skin, dull, rough or muddy skin, acne or seborrheic dermatosis. Hyper-seborrheic oily skin is characterized by exaggerated secretion and excretion

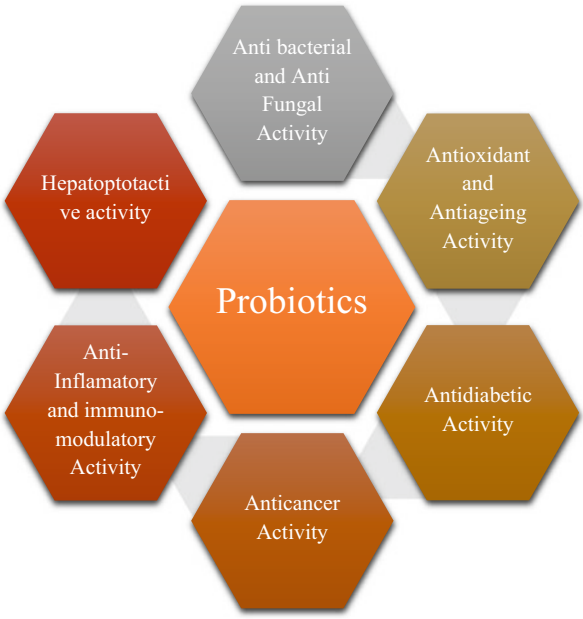

Fig. 15.6 Pharmaceutical application of probiotics

of the sebum resulting in the skin which may contribute to acne vulgaris (Li et al. 2019). The action of bacteria lipase converts varying parts of the triglycerides so as to give free fatty acids. These bacteria acquire energy by metabolism fatty acids in the sebum resulting in the injury to the sebaceous gland lining. Probiotic stimulates the synthesis of proteins capable of promoting antimicrobial defense of the epidermis and reestablish the ecoflora on the skin resulting in the decrease in the excessive sebum secretion (Castiel and Gueniche 2008; Gueniche et al. 2017).

Tropical cleaning composition for restoring the natural bacterial balance of the skin was developed using spore-forming bacteria like *Bacillus coagulans, Bacillus subtilis,* or *Lactobacillus sporogenes*. *Serratia marcescens* and *Enterococcus faecalis* were utilized to evaluate the ability to exterminate number of transient bacteria than normal flora (A detergent composition comprising probiotics/prebiotics active ingredient 2017).

Antibacterial cosmetic composition containing triclosan or benzalkonium chloride effects both beneficial and harmful bacteria present on the skin and excessive use of such formulation weakens the natural defenses of the skin leaving it more vulnerable to the pathogens. *Staphylococcus epidermidis* is the natural bacteria on the skin synthesizing various enzymes that rejuvenate physiological renewal mechanism of the skin even during aging. Composition containing various strains of bifidobacteria having selected antibacterial activity can be capable of treating and maintaining the firmness, resistance, and natural mechanism of the renewal and defense of the skin (De Miranda Chaves Vasquez Pinto 2016). Similarly, probiotics like ascomycetes, Staphylococcus, Peptostreptococcus, Leuconostoc,

Table 15.4 Beneficial health effects of probiotics and their mechanism of actions

Sr. No.	Health effects	How it works?	Promising bacterial strain	References
1	Skin dysbiosis and Eczema	Improving atopic eczema, atopic dermatitis, healing of burn and scars, rejuvenating the skin and also improving the skin's innate immunity	*Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, and *Firmicutes*	Baquerizo et al. (2014)
2	Antidiabetic effects	Improves low grade chronic inflammation, Useful in Insulin Sensitivity, Stimulate gut hormones, delayed the onset of glucose intolerance the gut microbiota–butyrate–inflammatory axis	*Lactobacillus acidophilus* and *Lactobacillus casei*	Yadav et al. (2007)
3	Lactose intolerance	Reduced level of lactose in fermented products by conversion of Lactic acid and presence of Lactase enzyme	*B. animalis* *L. paracasei* *B. animalis* ssp. *lactis* BB12 *L. acidophilus* NFCM *L. johnsonii* La1	Zhong et al. (2006)
4	Anti-Diarrheal effects	Antagonistic action against infectious enteric pathogens, limiting access of enteric pathogens (pH, bacteriocins/ defensins, antimicrobial peptides, Lactic acid production, and toxic oxygen metabolites)	*Lactobacillus* GG *Lactobacillus plantarum* *Lactobacillus casei* DN-114 001	Liu et al. (2017)
5	Anti-inflammatory effect	Reduction of pro-inflammatory molecules in body	*Bifidobacterium longum* 536, *L. paracasei* CNCM I-4034, *B. breve* CNCM I-4035, and *L. rhamnosus* CNCM I-4036	Plaza-Díaz et al. (2017)
6	Antitumor/ Anticarcinogenic	Degradation of precarcinogenic compounds and prevent conversion to Carcinogens,	*B. lactis* LKM 512 *L. casei* *L. rhamnosus* *L. rhamnosus* GG, *bifidobacteria*,	Hayatsu and Hayatsu (1993) and El-Nezami et al. (2000)

(continued)

Table 15.4 (continued)

Sr. No.	Health effects	How it works?	Promising bacterial strain	References
		Alternation of pro-cancerous activity of colonic microbes, Antimutagenic activity, Production of polyamines, Decrease in the carcinogenic aflatoxin in the lumen, reduced the fecal levels of enzymes that convert precarcinogens to carcinogens	*L. rhamnosus* GG, *L. rhamnosus* LC-705, and *Propionibacterium* sp.	
7	Immunomodulation	Stimulation of Immune system, Strengthening of non-specific and antigen specific defense against infection and tumors	*Lactobacillus* and *Bifidobacterium*	Bodera and Chcialowski (2009)
8	Anti-allergic effects	Degrade allergens and prevent translocation of Ag into blood streams, stimulate systemic, cell-mediated immunity, enhanced antigen elimination, downregulated inflammatory responses via direct effects on the immune system, modified degradation, permeation and presentation of food antigens, generation of pro-inflammatory cytokines	*Bifidobacterium longum* BB 536 *Lactobacillus rhamnosus* HN001 *Bifidobacterium animalis* subsp. lactis strain HN019, etc.	Harish and Varghese (2006) and Kaila et al. (1995)
9	Improved Inflammatory Bowel Disease (IBD)/ Crohn's disease	Reduced inflammation, Improved intestinal barrier function particularly tight junctions, Normalization of a	*Klebsiella* sp. *Lactobacillus plantarum* *Bifidobacterium infantis* 35624 *L. rhamnosus* GG, *L. rhamnosus* LC	Niedzielin et al. (2001)

(continued)

Table 15.4 (continued)

Sr. No.	Health effects	How it works?	Promising bacterial strain	References
		small bowel microflora reduced the ratio of anti- to pro-inflammatory cytokines	705, *B. breve* Bb 99, and *P. freudenreichii* subsp. *shermanii* JS	
10	Hypocholesterolemic (Improved Cardiac Health) effect	Inhibition of Cholesterol synthesis by bile salt deconjugation activity, Reduced cholesterol level by cholesterol assimilation by probiotic organisms	*Enterococcus faecium* M-74 *Lactobacillus plantarum Propionibacterium freudenreichii Lactobacillus plantarum* PH04	Hassan et al. (2019)
11	Antihypertensive effect	Bacterial peptidase action on milk protein results in antihypertensive tri-peptides, Cell wall components act as ACE inhibitors	*B. longum*, *B. infantis*, *B. breve*, *B. animalis*, *Bifidobacterium thermophyllum*, *L. acidophilus*	Fabian and Elmadfa (2006)
12	Prevent Urinogenital Infections	Competitive exclusion by adhesion to urinary and vaginal tract cells, Inhibitors production (H_2O_2, biosurfactant)	*Lactobacillus rhamnosus* GR-1 *Lactobacillus reuteri* RC-14 *Lactobacillus rhamnosus* GR-1 *Lactobacillus reuteri* RC-14	Anukam et al. (2009)
13	Coronary heart disease	Assimilate cholesterol induced a significant elevation in the mean HDL cholesterol level resulting in a significant improvement of the total/HDL cholesterol ratio	*B. longum*, *B. infantis*, *B. breve*, *B. animalis*, *Bifidobacterium thermophyllum*, *L. acidophilus*	Fabian and Elmadfa (2006) and Tahri et al. (1996)

Fusobacterium were explored with the hesperidin and its derivative to strengthen the skin barrier function (Gueniche and Castiel 2008).

Lysate of bifidobacterium species can employed for treating the body odor by degrading, inhibiting, or modifying the bacteria involved in the degradation of sweat/sebum along with the antiperspirant agent (Castiel et al. 2010). The lysate of the same genus of bacteria is useful in treating the oily scalp and re-establishing a

balanced ecoflora on the oily scalp. This lysate and some of the lactobacillus species also possess antipruritic, anti-inflammatory, and antidandruff activity to restore antimicrobial defenses and treating seborrheic dermatitis of oily scalp. This formulation for scalp disorder can be formulated as lotions, scalp care milk/gel, oral tablets, powder, sticks, or shampoo (Gueniche et al. 2008; Breton et al. 2007).

Bifidobacterium and its lysate can also be employed to prevent and treat skin disorder, skin irritation in the sensitive skin, in the form of aqueous, aqueous-alcoholic or oily solutions or dispersions in the form of lotions, o/w, w/o emulsion, serum or semi-solid milk (Gueniche 2010). Similarly probiotics in the form of lactobacillus or bifidobacterium or its metabolites are claimed to reduce skin irritation caused by stimulation of interleukin and cytokinins. *Lactobacillus paracasei* in food and drinks are claimed to be useful for prevention and treatment of allergy (Kirin Holdings Co Ltd 2004).

A highly skin friendly mask essence with the fast permeation with effective skin whitening property comprised of natural probiotic active ingredients of Saccharomyces Leuconostoc, Lactobacillus bifida, Acidophilus lysate, or grape fermentation products along with the PEG-40 hydrogenated castor oil, flavor, preservatives, stabilizing and penetration enhancing agents (Probiotic whitening recovery mask essence and preparation method thereof 2015).

Gastrointestinal inflammation, rheumatoid arthritis, osteoarthritis, autoimmune inflammatory disease, skin conditions like psoriasis, dermatitis, eczema, and onychomycosis were claimed to be treated by prebiotic *Lactobacillus parafarraginis*, *L. buchneri, L. rapi, L. zeae, Acetobacter fabarum, Candida ethanolica*, or their cell free filtrates as described in the patent WO2018187838A1 (Finlayson 2018).

The color cosmetic emulsion or powder with probiotic Lactobacillus, lamellar phyllosilicate mineral, Citrus genus with anti-inflammatory activity along with other formulatory additives can be used as foundation makeup, blush, eyeshadows, concealer, or lipstick, also soothing irritation, inflammation, and normalization of skin. The other probiotic like *Saccharomyces ferment* lysate, *Lamaralia saccharina* ferment along with apple ginseng, garlic, or grape ferment improved the performance of the formulation (Dao et al. 2010).

A hair color product containing probiotic from yogurt was patented to moisturize, soften, condition, straighten, strengthen, and repair hair, in addition to promote healthy scalp. The probiotic group includes various species of Lactobacillus, Bifidobacteria, Bifidobacteria, Pediococcus, and Lactococcus. Conditioning agent comprises 80% PEG 200, 1% glyceryl stearate as emulsifier, and 5% glycerine as humectant accompanied with milk solid and whey protein concentrate (Dao et al. 2010; Albano 2004).

Bifidobacterium lysate contains many enzymes like lactic acid dehydrogenase, phosphatase, phosphoketolase, and transaldolase along with cell wall component like peptidoglycan, mucopeptide, teichoic acid, etc. which can be used for dermalogical or cosmetic application to slow down skin aging. The dosage form can be applied topically in the form of cream, ointment, lotion, serum, or foam to improve hydration, cutaneous homeostasis, and treatment of signs of aging like

wrinkles, fine line, loss of firmness, elasticity, or tonicity of the epidermis (Amar et al. 2010).

Probiotic living cells along with the prebiotics can be encapsulated to preserve their viability and health benefits. Such microcapsule successfully delivers probiotics through the hostile stomach milieu to the lower gastrointestinal tract. Microcapsules are formed from the natural polymers like plant based protein such as chickpea protein, soya protein and biopolymers such as alginate, carrageenan, or gellan gum when subjected to cross linking or by ionic gelation technique (Patheson and Burkholder 2003; Nickerson et al. 2014).

Alternate method to stabilize viable probiotic prebiotic products is lyophilization, in presence of cryoprotectant to be used as pharmaceuticals, food, or cosmetic additive. The cryoprotectant protects cells viability during freeze-drying process and includes skimmed milk, dimethyl sulfoxide, glycerol, formamide, methyl acetamide, PVP, propylene glycol, serum albumin, alginates, or carbohydrates like sucrose, glucose, lactose, raffinose, dextrin, pectin, or cellulose sulfate (Guenzburg et al. 2014).

In patent WO 20131818626A2 stabilized probiotic aqueous formulation with an oxygen carrying compound, biocompatible binder was formulated as personal care product. The biocompatible binder consists of semi-synthetic cellulose derivative, gellan gum, or alginate. Probiotic along with the above ingredients were encapsulated in microspheres or micro pellets using fluidized bed coating method (Liang et al. 2013).

The vaginal probiotic product comprising dried *Lactobacillus casei/gasseri*, boric acid, vitamin E with the carrier maltodextrin in the capsule shape dosage form, inserted intravaginally for vaginal atrophy, dryness, bacterial vaginosis, yeast infection, vaginosis, or vaginal bleeding. Additionally, guar gum, citric acid or aloe vera extract in the powdered form aids in maintaining vaginal pH and improvement in efficacy. This product is claimed to be useful in the post-surgical or childbirth trauma and to improve, maintain, or achieve vaginal health (Krebs-Bensch 2017).

15.7 Regulatory Challenges in Probiotics

Probiotics are available as foods and dietary supplements for long time. Initially marketed in yogurts and dairy products, the use of probiotics in commercial products has increased rapidly in recent years. Other probiotic products including juices, nutrition bars, infant formulas, relishes and condiments, sweeteners, waters, pizza crust, gum, lozenges, dietary supplements, toothpaste, and cosmetics are growing vastly in the market (Hoffmann et al. 2014).

Probiotics are available in foods and dietary supplements, even as pharmaceutical formulations (capsules, tablets, and powders) and in some other forms as well, but their claims of health benefits may challenge the traditional border between food and medicine. A number of probiotic products have been already introduced into the international market as food supplements, dietary supplements, natural health products, functional foods, and many more other categories; as a result, the position

of regulatory system for probiotics within existing categories become vague and quite unclear. The lack of a consistent terminology across the globe leads to legal uncertainty and confusion instead of being a direct obstacle for development of a mature market.

While probiotics fall into virtually every product category regulated by the FDA. It does not have a central office or pathway that deals specifically with probiotics. Nor does the agency have a regulatory definition of probiotics. When questions arise regarding into which category a probiotic belongs, the answer is determined on a case-by-case basis. Classification of probiotics as a drug triggers the extensive and costly Investigational New Drug application (IND) process, which typically includes Phase I, II, and III clinical trials. Foods and dietary supplements do not require agency premarket approval. The current regulatory framework does not address the role of foods in treating, mitigating, or curing disease. Probiotics fall into multiple product categories; this may lead to inter-center inconsistencies in interpretation and application of regulations, data requirements, and the content of potentially relevant guidance documents about probiotics (Saldanha 2008).

Probiotics are categorized under different categories in different countries. They are named differently as natural health products in Canada, dietary supplements, drugs, medical food, live bio-therapeutic agent, biological agent as per their intended use in USA, functional food in Japan, China, Malaysia, as food supplement in Sweden, Denmark, and Finland, bio-therapeutic/pharmaceuticals European countries like Belgium and Germany. Probiotics are not considered as single category rather subcategorized under different categories and are defined separately by different countries given in Table 15.5.

Probiotic products have been commercialized and made their importance within past years. Due to constant reporting of novel strains due to their novel health benefits, safety and efficacy issues of these products must be carefully resolved before their use into our own food. Improper use of the term probiotics should be rectified because lack of awareness and the credibility of health claims associated with probiotic products are posing a major threat to probiotic industry and its consumers. In this situation a common regulatory framework is required which will allow free exchange of products and will minimize confusion of different regulations. Which will be later helpful to bring harmonized guidelines into consideration for future safe and efficacious use of probiotics.

15.8 Future Directions

The development of successful probiotic products will be contingent on both proof of probiotic effect and development of foods that harbor high number of viable organisms at the time of consumption. Incorporation of prebiotic and probiotic in various oral and cosmetic formulation is now not a new concept; however, complete understanding of where, when, and how to use them along with its precise mechanism of action is still to be established scrupulously. Consequently, continued scientific endeavor with the aim to understand at a cellular and molecular level,

Table 15.5 Countrywide regulatory category for probiotics

Sr. No.	Country	Category	Regulatory body
1	General	Probiotics	FAO/WHO
2	Japan	Functional foods and Nutraceuticals	MHLW/FOSHU
3	Europe	Functional foods	FUFOSE
4	China	Functional foods	SFDA
5	Brazil	Functional foods	ANVISA
6	New Zealand and Australia	Functional foods	FSANZ
7	USA	Dietary supplements	DSHEA
		Drugs	FDA
		Biological products	BLA
		Medical food	FDA
		Live bio therapeutic agent	FDA
8	India	Functional foods and Drugs	FSSA, PFA and FDA
9	Malaysia	Functional food	FSQD
10	Canada	Natural health product	Natural health product directorate

Where, FAO/WHO = Food and Agricultural Organization/World Health Organization, MHLW = Ministry of Health and Welfare, FOSHU = Food for Specified Health Use, FUFOSE = Functional Food Science in Europe, SFDA = State Food and Drug Administration, ANVISA = National Health Surveillance Agency Brazil, FSANZ = Food Standards Australia and New Zealand, DSHEA = Dietary Supplement Health and Education Act, BLA = Biologic License Application, PFA = Prevention of Food Adulteration Act, FSQD = Food Safety and Quality Division, NPCB = National Pharmaceutical Control Bureau

the health promoting mechanism of the probiotic's cultures must be seen as a crucial requirement for serving the future of probiotic.

Probiotics can be utilized not only in the food and nutraceuticals, products, cosmetics, and veterinary products, but their scope is now even extended as a medical nutrition for promoting rapid recovery in the surgical and trauma patients. However there exist some safety issues which need to be tackled vigilantly. Probiotics differ greatly in their effects on health; hence accurate identification of microorganisms to the strain level is required. The risk and propensity to transfer genetic material from the pathogen to probiotics in vivo making fruitful microbes' threat to health need to be studied thoroughly. Food, nutraceuticals, and cosmetic probiotic products utilized must be differentiated from drug like probiotic preparations prescribe for specific clinical indications and subjected to rigorous clinical trials for their respective applications. Quality control standardization and validation control need to be developed for this products manufacturing under the patronage of respective regulatory agencies.

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Saba Haq and Naresh Poondla

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